1.0 INTRODUCTION

1.1 PURPOSE OF THIS DOCUMENT

This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) to protect human health, pursuant to Section 304(a) of the CWA. Under the CWA, States and authorized Tribes are to establish water quality criteria to protect designated uses. While this document constitutes the U.S. Environmental Protection Agency's (EPA's) scientific recommendations regarding concentrations of methylmercury in fish and shellfish that protect human health, this document does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and may not apply to a particular situation based upon the circumstances. State and Tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. EPA may change this guidance in the future.

This document establishes a water quality criterion for methylmercury. The U.S. Environmental Protection Agency (EPA) originally published an Ambient Water Quality Criterion (AWQC) for total mercury in 1980. That AWQC was partially updated in 1997 to incorporate a change in the reference dose (RfD). As required under Section 304(a) of the Clean Water Act, EPA must periodically revise criteria for water quality to accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on human health from the presence of pollutants in any body of water. The criterion uses new methods and information described in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000) (2000 Human Health Methodology) and in the Methodology's accompanying *Federal Register Notice* (U.S. EPA, 2000a,b). These new methods include updated approaches to determine toxicity dose-response relationships for both carcinogenic and noncarcinogenic effects, updated information for determining exposure factors, and new procedures to determine bioaccumulation factors.

Development of a methylmercury criterion involves some unique considerations compared with many of EPA's past efforts in the water quality criteria program. Traditionally, EPA has established recommended 304(a) criteria to protect human health as ambient concentrations in water. For those pollutants that bioaccumulate, such as methylmercury, exposure through the food pathway is estimated by using a bioaccumulation factor (BAF). However, following review of available data and

recommendations made by external peer reviewers (U.S. EPA, 2000c), EPA determined that it is more appropriate to base the methylmercury criterion on a fish tissue residue concentration than on an ambient water concentration. This determination was partly based on the current scientific understanding of the fate of mercury and methylmercury in aquatic ecosystems. Another factor was the limited information on sources of mercury and the conversion to methylmercury (and its bioavailability). Additional considerations were the difficulty in measuring methylmercury in the water column and relating it to concentrations in aquatic organisms. EPA believes that the latest data and science on methylmercury exposure, effects, and environmental fate support the derivation of a fish tissue residue criterion.

1.2 PRIMARY DATA SOURCE

Much of the information in this document has been taken from the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997b-h). This comprehensive, eight-volume study was prepared by EPA and submitted to Congress in 1997 to fulfill the requirements of section 112(n)(1)(B) of the Clean Air Act, as amended in 1990. The MSRC provides an assessment of the magnitude of U.S. mercury emissions by source, the health and environmental implications of those emissions, and the availability and cost of control technologies. As the state of the science for methylmercury continues to evolve, information from the MSRC has been supplemented by data and analyses published since 1997. The health effects information used in the derivation of the reference dose (RfD) for the fish tissue residue concentration is based on the recommendations of the National Academy of Sciences National Research Council report, *Toxicological Effects of Methylmercury* (NRC, 2000). For additional discussion on the NRC recommendations, see Section 4 of this criteria document. The comments of the methylmercury RfD scientific peer review panel also guided the risk assessment.

1.3 CHEMICAL AND PHYSICAL PROPERTIES

The water quality criterion is being derived for methylmercury (CAS No. 22967-92-6). Synonyms for methylmercury include MeHg, methylmercury ion, methylmercury ion (1+), methylmercury (1+), methyl mercury, and methylmercury(I) cation (Prager, 1997). A commonly occurring form of methylmercury is methylmercuric chloride ($CH_3Hg^+Cl^-$), a stable salt form that exists as a white crystal. This compound is often used in laboratory dosing experiments investigating the toxicological properties of methylmercury. Because methylmercury exists as a free ion only in minute quantities (Prager, 1997), the chemical and physical data provided below are for the chloride salt.

The table below presents available chemical and physical data for methylmercuric chloride (ATSDR, 1999; Kaufman, 1969).

Chemical formula	CH_3HgCl
Chemical structure	$CH_3—Hg^+ Cl^-$
Molecular weight	251.10 (g/mol)
Physical state (25°C)	White crystal
Boiling point (at 25 mm Hg)	No data
Melting point	170°C
Density (25°C)	4.06 g/mL
Vapor pressure (25°C)	0.0085 mm Hg
Water solubility (21°C)	<100 mg/L
Log octanol/Water partition coeff.	No data
Odor threshold (air)	No data
Conversion factors (air)	1 ppm = 10.27 mg/m^3; 1 mg/m^3 = 0.0974 ppm

2.0 TOXICOKINETICS

This section presents information on the absorption, distribution, metabolism, and excretion of methylmercury in humans and animals. This information is summarized from Volume V, Chapter 2 of the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997e).

2.1 ABSORPTION

2.1.1 Oral Absorption

Methylmercury is efficiently absorbed from the gastrointestinal tract following ingestion. Approximately 94%-95% of methylmercury in fish ingested by volunteers was absorbed from the gastrointestinal tract (Aberg et al., 1969; Miettinen, 1973). Aberg et al. (1969) found uptake of greater than 95% of radiolabeled methylmercuric nitrate administered in water to human volunteers.

Data from studies on rats, cats, and monkeys support these absorption estimates (ATSDR, 1999). Studies on rats indicate rapid and complete absorption of inhaled methylmercury vapor into the bloodstream (Fang, 1980). Female cynomolgus monkeys administered 0.5 mg mercury per kilogram of methylmercuric chloride by oral gavage experienced complete absorption within 6 hours (Rice, 1989).

2.1.2 Absorption via Other Routes

Limited information is available on absorption via inhalation and dermal routes. There is one reported human dermal exposure when a 48-year-old chemistry professor inadvertently spilled drops (0.4-0.5 mL) of dimethylmercury from her pipette into her latex gloves. Penetration of dimethylmercury through the gloves occurred instantaneously. Mercury hair level was elevated to almost 1,100 ppm, with a half life of 74.6 days. Five months after exposure, the woman experienced severe neurotoxicity and died 9 months later (Blayney et al., 1997; Nierenberg et al., 1998).

Skog and Wahlberg (1964) evaluated the dermal absorption of the methylmercuric cation in guinea pigs. The test material was applied as the dicyandiamide salt. Absorption was estimated by disappearance of the applied compound and by appearance of mercury in kidney, liver, urine, and blood. Approximately 3% to 5% of the applied dose was absorbed during a 5-hour period.

Indirect evidence in animals indicates that inhaled methylmercury vapor is absorbed readily through the lungs. Fang (1980) showed a correlation between tissue mercury levels and both exposure level and exposure duration in rats exposed to radioactively labeled methylmercury vapor. The percent absorbed was not quantified.

2.2 DISTRIBUTION

After absorption from the gastrointestinal tract, methylmercury is readily absorbed into the blood and distributes to all tissues, including the brain and fetus. The fraction of the absorbed dose that is found in the blood has been estimated in three studies. Kershaw et al. (1980) reported an average fraction of 0.059 of the absorbed dose in total blood volume, based on a study of five adult male subjects who ingested methylmercury-contaminated tuna. In a group of nine male and six female volunteers who had received ^{203}Hg-methylmercury in fish, approximately 10% of the total mercury body burden was present in 1 L of blood in the first few days after exposure; this dropped to approximately 5% over the first 100 days (Miettinen et al., 1971). Sherlock et al. (1984) derived an average value of 1.14% for the percentage of absorbed dose in 1 kg of blood from data on subjects who consumed a known amount of methylmercury in fish over a 3-month period. Average daily intake in the study ranged from 43 to 233 µg/day. There was a dose-related effect on percentage of absorbed dose that ranged from 1.03% to 1.26% in 1 L of blood. Each of these values was multiplied by 5 to yield the total amount in the blood compartment, as there are approximately 5 L of blood in an adult human body.

Methylmercury in the blood is found predominantly in the red cells (Kershaw et al., 1980; Thomas et al., 1986). It is distributed throughout the body following absorption from the gastrointestinal tract into the blood (Clarkson, 1972; Hansen, 1988; Hansen et al., 1989; Nielsen and Andersen, 1992; Soria et al., 1992; Suzuki et al., 1984). Although the distribution of methylmercury in the body is generally uniform, at least one animal study indicates that high levels can be found in the kidney. Rice (1989b) administered 0.025 or 0.05 mg mercury/kg-day as methylmercuric chloride in apple juice to cynomolgus monkeys for approximately 2 years. Kidney tissue concentrations of mercury ranged from 10 to 28 ppm in the cortex and 1 to 10 ppm in the medulla when assessed more than 200 days after cessation of treatment. In contrast, mercury concentration was less than 2 ppm in the other tissues evaluated.

Methylmercury easily penetrates the placental barrier in humans and animals (Hansen, 1988; Hansen et al., 1989; Nielsen and Andersen, 1992; Soria et al., 1992; Suzuki et al., 1984). Several studies

have demonstrated mercury in newborn cord blood. The relationship to maternal blood is variable (Grandjean et al., 1999). Information on this relationship is discussed in Section 4.5.4.1.

The distribution of methylmercury in animals may vary by age and sex (Thomas et al., 1982,, 1986, 1988). Female rats exposed to methylmercury had higher peak levels of mercury in the kidney (primarily as methylmercury) than males; inorganic mercury levels did not differ significantly between the sexes (Thomas et al., 1986). Accumulation of mercury was found to be higher in the bodies of neonatal rats (Thomas et al., 1988) than in adult rats (Thomas et al., 1982). Ten days after administration of methylmercury, 94% of the dose was still detected in neonates while approximately 60% was retained in adults (Thomas et al., 1988). The longer retention of mercury in neonates may result from multiple factors, including the high levels of mercury accumulated in the pelt of neonates owing to lack of clearance (Thomas et al., 1988) and the lack of a fully developed biliary transport system in neonates (Ballatori and Clarkson, 1982).

2.3 METABOLISM

The time required for methylmercury metabolism to inorganic mercury may account for the latent or silent period observed in epidemiological studies from methylmercury poisoning incidents in Japan and Iraq. During the latent period (both during and after the cessation of exposure) the patient feels no untoward effects. It is possible that a number of biochemical changes may take place in parallel during this period, and some may not be causatively related to the clinical outcome. Ganther (1978) hypothesized that the carbon-mercury bond in methylmercury undergoes homolytic cleavage to release methyl free radicals. The free radicals are expected to initiate a chain of events involving peroxidation of lipid constituents of the neuronal cells. The onset of symptoms is delayed for the period of time that cellular systems are able to prevent or repair effects of lipid peroxidation. When the cellular defense mechanisms are overwhelmed, rapid and progressive degeneration of the tissue results. In the Iraqi poisoning incident, the latent period before toxic signs were noted varied from a matter of weeks to months. In contrast, the latency observed in the Japanese poisoning incident was as long as a year or more. The difference in duration may in part be due to the presence of selenium in the fish ingested by the Japanese population.

Rat liver microsomes can metabolize methylmercury into inorganic mercury via the NADPH-cytochrome P-450 reductase, also known to control hydroxyl radical production in liver microsomes (Suda and Takahashi). To a lesser degree, an oral dose of methylmercuric chloride may also be

converted into inorganic mercury via the intestinal flora (Nakamura et al, 1977; Rowland et al., 1980). The intestinal wall is poor in absorbing the inorganic mercury, thus almost all of it is excreted. Studies in mice appear to indicate that toxicity from exposure to dimethylmercury results from the biotransformation of dimethylmercury to methylmercury (Ostland, 1969). Following acute exposure to methylmercury, most of the mercury in the brain is in the organic form; however, with chronic exposures, a greater amount is in the inorganic form, suggesting that the rate of demethylation increases with long-term exposure (Aschner and Aschner, 1990). Rice (1989a, 1989) demonstrated that tissue half-life of methylmercury in the brain may be significantly longer than the blood half-life.

In rats, methylmercury in the body is relatively stable and is only slowly demethylated to form mercuric ion (Norseth and Clarkson, 1970). The demethylation appears to occur in tissue macrophages (Suda and Takahashi, 1986), intestinal microflora (Nakamura et al., 1977; Rowland et al., 1980), and fetal liver (Suzuki et al., 1984).

2.4 EXCRETION

In humans, approximately 90% of the absorbed dose of methylmercury is excreted in the feces (U.S. EPA, 1997e). Excretion via the urine is relatively minor but slowly increases with time; at 100 days after dosing, urinary excretion of mercury accounted for 20% of the daily amount excreted. The urinary excretion of mercury may reflect the deposition of demethylated mercury in the kidneys and its subsequent excretion. In humans the major routes of excretion are via the bile and feces.

Feces are also the predominant route of methylmercury elimination in adult animals (Farris et al., 1993; Hollins et al., 1975; Thomas et al., 1987). Biliary excretion of methylmercury and its demethylation in gastrointestinal flora have been reported in rats (Farris et al., 1993). After a single oral dose of methylmercury, the major elimination route was the feces (65% of the administered dose as inorganic mercury and 15% of the administered dose as methylmercury) and the minor route was urine (1% of the administered dose as inorganic mercury and 4% of the administered dose as methylmercury) (Farris et al., 1993). Following administration of methylmercuric nitrate, 33% of the administered dose was excreted in 49 days; 0.18% to 0.27% excretion in the urine in 10 days and 3.3% urinary excretion in 49 days. This continued for up to 71 days postingestion (Miettinen, 1973). Forty to 50 days postingestion, <0.12% of the administered dose of mercury was found per gram of hair. The half-life for methylmercury appeared to be 70-74 days. In humans the whole body half-life of methylmercury was estimated to be between 70 and 80 days (Aberg et al., 1969; Miettinen, 1973; Bernard and Purdue, 1984).

Mercury is excreted into the hair of methylmercury-exposed humans and animals. Incorporation of mercury into hair is irreversible, and hair analysis is thus a useful tool for monitoring exposure to methylmercury. Segmental analysis of hair may be used to provide a historical record of exposure patterns.

Methylmercury is excreted in breast milk (Bakir et al., 1973; Sundberg and Oskarsson, 1992). The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to methylmercury via contaminated grain in Iraq between 1971 and 1972 (Bakir et al., 1973). Evidence from the Iraqi poisoning incident also showed that lactation decreased blood mercury clearance half-times from 75 days in males and nonlactating females to 42 days in lactating females; the faster clearance due to lactation was confirmed in mice (Greenwood et al., 1978). In mice, of the total mercury in the breast milk, approximately 60% was estimated to be methylmercury. Skerfving (1988) has found that 16% of mercury in human breast milk is methylmercury. Studies in animals indicate that the mercury content of breast milk is proportional to the mercury content of plasma (Sundberg and Oskarsson, 1992; Skerfving, 1988).

In rat and monkey neonates, excretion of methylmercury is severely limited (Lok, 1983; Thomas et al., 1982). In rats dosed prior to 17 days of age, essentially no mercury was excreted (Thomas et al., 1982). By the time of weaning, the rate of excretion had increased to adult levels. The failure of neonates to excrete methylmercury may be associated with the inability of suckling infants to secrete bile (Ballatori and Clarkson, 1982) and the decreased ability of intestinal microflora to demethylate methylmercury during suckling (Rowland et al., 1977).

Currently, five studies report clearance half-lives for methylmercury. Three studies suggest a half-life of approximately 70 to 80 days (Aberg et al., 1969; Bernard and Purdue, 1984; Miettinen, 1973). Smith et al. (1994) reported a half-life of 44 days in a study of seven adult males treated intravenously with methylmercury. In this study, methylmercury and inorganic mercury concentrations in blood and excreta were determined separately based on differential extractability into benzene. The predominant species in the blood was methylmercury; there was no detectable methylmercury in the urine. Al-Shahristani and Shihab (1974) calculated a “biological half-life” of methylmercury in a study of 48 male and female subjects who had ingested seed grain contaminated by organic mercurials. The half-life, determined from distribution of mercury along head hair, ranged from 35 to 189 days with a mean of 72 days.

The relatively long half-life of methylmercury in the body results partly from reabsorption of methylmercury secreted into the bile (hepatobiliary cycling) (Norseth and Clarkson, 1971). In this cycle, methylmercury forms a complex with glutathione in the hepatocyte and the complex is secreted into the bile via a glutathione carrier protein (Clarkson, 1993). The methylmercury-glutathione complex in the bile may be reabsorbed from the gallbladder and intestines into the blood. This cycle is terminated when intestinal microorganisms demethylate methylmercury to form mercuric ion (Rowland et al., 1980). Mercuric mercury is poorly absorbed from the intestines and the fraction that is not reabsorbed is excreted in the feces. As noted above, approximately 90% of the absorbed dose of methylmercury is ultimately excreted in the feces as mercuric mercury.

2.5 BIOLOGICAL MONITORING

Distribution of methylmercury to hair and blood provides a means for biological monitoring of methylmercury exposure. This section provides an overview of the use of hair and blood for assessing exposure and outlines the available methods for quantitation.

2.5.1 Blood

Methylmercury distributes freely throughout the body, and thus blood is a good medium for estimating short-term exposure. Blood levels may not necessarily reflect methylmercury intake over longer periods, as an individual's intake may fluctuate (Sherlock et al., 1982; Sherlock and Quinn, 1988).

The characteristic partitioning of mercury in the blood permits identification of the form of mercury to which an individual has been exposed. Measurements of blood hematocrit and mercury concentrations in both whole blood and plasma can be used to calculate the red blood cell to plasma mercury ratio. In the case of methylmercury, examination of this ratio enables estimation of interference from exposure to high levels of elemental or inorganic mercury (Clarkson et al., 1988).

2.5.2 Hair

Scalp hair is a useful indicator for estimating methylmercury exposure (Phelps et al., 1980). Mercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood. The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as μg mercury/g hair to mg mercury/l blood. Uncertainty in measurements, interindividual variation in body burden, differences

in hair growth rates, and variations in fresh and saltwater fish intake have led to estimates ranging from 190:1 to 370:1 and higher (Birke et al., 1972; Skerfving, 1974; Phelps et al., 1980; Turner et al., 1980; Sherlock et al., 1984). Once incorporated into the hair, the mercury is stable, and can give a longitudinal history of blood methylmercury levels (Phelps et al., 1980; WHO, 1990). The identity of the predominate chemical species (inorganic or methylmercury) depends on exposure patterns and the extent of methylmercury demethylation.

Chemical analyses to determine mercury content of hair assay total mercury rather than chemical species of mercury. As a result, the fraction of hair mercury that is methylmercury is an estimate based on knowledge of environmental and occupational exposure patterns (U.S. EPA, 1997f). Analysis of hair mercury levels may be confounded by several factors, including adsorption of mercury vapor onto the hair strands, natural hair color, hair treatment, and growth rate (Francis et al., 1982; Suzuki, 1988).

Analysis of mercury in maternal hair has been utilized to estimate the fetal burden. This approach has been validated by Cernichiari et al. (1995), who collected blood samples and autopsy brains from terminally ill neonates in a population exposed to methylmercury via fish consumption. Maternal blood and hair samples were also obtained. The concentrations of total mercury in six major brain regions of the neonates were highly correlated with the concentration of mercury in a 1-cm segment of maternal hair next to the scalp (correlation coefficients 0.6 to 0.8, $p<0.01$). These correlations were confirmed by a series of comparisons utilizing maternal hair, maternal blood, neonate blood, and neonate brain tissue.

2.5.3 Methods of Analyzing Mercury Concentrations in Biological Samples

The most common methods used to determine mercury levels in biological media include atomic absorption spectrometry, neutron activation analysis, X-ray fluorescence, and gas chromatography. Another method is anodic stripping voltammetry (Liu et al., 1990). Gas chromotography-electron capture is the only method capable of differentiating methylmercury from other species, whereas cold vapor atomic absorption spectrometry will detect mercury at parts per billion in both urine (Magos and Cernik, 1969) and blood samples (Magos and Clarkson, 1972). Mercury content in hair has been measured by cold vapor atomic absorption spectrometry, atomic fluorescence spectrometry, X-ray fluorescence, and neutron activation analysis (Zhuang et al., 1989).

Another method for analyzing biological samples containing methylmercury is with the use of *Pseudomonas putida* strain FB1. The method is considered very reliable and specific for methylmercury

quantification because chemical inference is negligible. The *Pseudomonas putida* bacteria is capable of converting methylmercury to methane gas and elemental mercury (Baldi and Filippelli, 1991), thus allowing the detection of 15 ng of methylmercury in 1 g of biological tissue with a coefficient of variation of 1.9%.

New methods, such as inductively coupled plasma-mass spectrometry (Kalamegham and Ash, 1992) for analyzing mercury in biological samples are being developed, but are considered very costly and unaffordable by many laboratories. For additional detail on other methods, please refer to the Toxicological Profile for Mercury (Update) (ATSDR, 1999) and in the World Health Organization (WHO) report Methylmercury (IPCS, 1990).

2.6 PHARMACOKINETIC MODELS

A number of extrapolations are generally required in risk assessments, including high-dose to low-dose extrapolations, route-to-route extrapolations, cross-species extrapolations, and extrapolations for varying exposure durations. Physiologically based pharmacokinetic (PBPK) modeling can increase the accuracy of these extrapolations if one has data to use in the model parameters. (Clewell and Andersen, 1985, 1989; Clewell, 1995a; Andersen et al., 1995).

For methylmercury, PBPK modeling in the risk assessment process is used to estimate the relationship between the measure of exposure used in epidemiological studies (mercury in hair and blood) and the daily ingested dose used to determine a reference dose. Several human PBPK models have been developed (Luecke et al., 1994, 1997; Smith et al., 1994; Gearhart et al., 1995; Clewell et al., 1999) to address this issue. Two animal models (Farris et al., 1993; Gray, 1995) were also developed to describe the disposition and metabolism of methylmercury and its major metabolite, mercuric mercury, in rats. A brief description of the pharmacokinetic models developed for methylmercury is presented here.

A PBPK model was developed by Farris et al. (1993) to simulate the disposition of methylmercury and its primary metabolite, inorganic or mercuric mercury, in the adult rat. Farris et al. (1993) also conducted metabolism and distribution studies in rats to collect the data needed to understand the processes that influence the pharmacokinetics of both methylmercury and mercuric mercury. This model incorporated time-dependent compartment volume changes, compartment volume-dependent clearance rates, and the recycling of mercury as a result of hair ingestion during grooming. The Farris model served as the foundation for several subsequent models developed for methylmercury.

On the basis of the modeling results reported by Farris et al. (1993), Smith et al. (1994) developed a simple human PBPK model. Smith et al. (1994) assumed that methylmercury behaved as a single pool while the behavior of its metabolite (inorganic mercury) varied in different tissues. Smith et al. (1994) also conducted experimental studies in human volunteers to monitor levels of methylmercury and inorganic mercury in the blood, urine, and feces following a single intravenous injection of a tracer dose of methylmercury. The modeling results indicated that inorganic mercury accumulated in the body and was the predominant form of mercury present at longer times following administration. The biological half-life of methylmercury in the body was estimated to be 44 days, with an estimated 1.6% of the body burden excreted each day.

Gray (1995) developed a PBPK model for methylmercury in the rat that could be used to evaluate the developmental toxicity observed following *in utero* exposure to methylmercury. The model consists of a maternal model with a fetal submodel. This model can be used to obtain fetal and maternal organ methylmercury concentration-time profiles for any maternal dosing regimen, including the dosing patterns used in rat developmental neurobehavioral studies.

Luecke et al. (1994) developed a generic PBPK model for human pregnancy that was applied (Luecke et al., 1997) to both rat and human kinetic data for methylmercury. This model consists of four submodels and incorporates the changes observed in both the mother and the fetus during the time course of pregnancy. Both rat and human data have been simulated using the model following various routes of exposure to methylmercury.

Stern (1997) identified data on the distribution of parameters in the one-compartment model from the published literature. Available data specific to women between the ages of 18 and 40 were used; data between men and women were also used to determine statistical differences, if any. Blood volume and body weight were assumed to be correlated. A similar approach was used by Swartout and Rice (2000). In that analysis, however, some of the parameters are described by different distributional shapes or by distributions from different data sources than those used by Stern (1997).

Swartout and Rice (2000) performed an uncertainty analysis of the estimated ingestion rates used to derive the methylmercury reference dose. The uncertainty arising from the calculation of ingestion dose levels in mg/kg per day corresponding to measured concentrations of mercury in hair is estimated through a Monte Carlo analysis of the EPA dose conversion model. The Monte Carlo model was modified to include a methylmercury elimination concentration that was converted to an equivalent half-

life, and a term was added to account for measurement error of hair-mercury concentrations. The authors assumed correlations between several pairs of parameters: the hair-to-blood ratio and the elimination-rate constant, body weight and blood volume, and the fraction of the absorbed dose in the blood and body weight. Applying the results of this analysis and assuming the input correlations to the benchmark dose of 11 ppm mercury in hair used in the derivation of the methylmercury RfD results in a lower 95% confidence limit of 4.07×10^{-4} mg/kg-day. The dose conversion factor simulation is 8.0×10^{-5} with a 90% confidence interval of 3.7×10^{-5} to 1.6×10^{-4}. The corresponding dose conversion value used in the derivation of the methylmercury reference dose is 9.8×10^{-5}. The 90% confidence interval spans a three fold to five fold range of ingestion doses for any given concentration of mercury in hair. The hair-to-blood mercury concentration ratio contributed to the variance of the output.

Gearhart et al. (1995) developed a multicompartment adult and fetal model to analyze epidemiological data for a methylmercury risk assessment. This model was recently reparameterized by Clewell et al. (1999) for use in a Monte Carlo variability and sensitivity analysis. The model structure, a modification of the model developed by Farris et al. (1993), consists of a maternal model with a fetal submodel. Changes in both maternal and fetal tissues during gestation are described. The model has the capability to estimate maternal hair and blood concentrations following ingestion of methylmercury, as well as the resulting fetal cord blood concentrations. This model was used to address the relationship between mercury in maternal hair and daily ingested dose, which has been identified as a major issue in conducting a risk assessment for methylmercury. The results of Monte Carlo analysis using the model provided an estimate of the variability in ingestion rates associated with a measured hair concentration. The predicted variability (ratio of median to 5th percentile equals 1.5) is comparable to similar analyses performed using a simple compartmental model (U.S. EPA, 1997e; Stern, 1997). The results of a sensitivity analysis of the model suggest that the most important determinants of pharmacokinetic variability for methylmercury are the hair:blood partition, body weight, and hair growth rate.

3.0 TOXICOLOGICAL BASIS FOR CRITERIA

This section of the *Water Quality Criteria for the Protection of Human Health* document for methylmercury relies heavily on information provided in the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997e) for summaries of studies published before 1997. Data published after 1997 are summarized in this chapter. The *Water Quality Criteria for the Protection of Human Health* document for methylmercury is not intended to be an exhaustive survey of the voluminous health effects literature available; rather, it includes detailed information on studies that form the basis for EPA's hazard identification and dose-response assessment. The database on neurodevelopmental effects of methylmercury is quite extensive. Developmental neurotoxicity is currently considered the most sensitive health endpoint. Data on cardiovascular and immunological effects are beginning to be published and may provide a more sensitive endpoint for low-dose methylmercury effects. This chapter will focus on developmental neurotoxic, cardiovascular, and immunological toxic effects of methylmercury exposure. The reader is referred to the MSRC for information on other toxic effects of methylmercury.

3.1 INTRODUCTION

Methylmercury is a highly toxic substance with a number of adverse health effects associated with its exposure in humans and animals. Human exposure following high-dose poisonings in Japan and Iraq resulted in effects that included mental retardation, cerebral palsy, deafness, blindness, and dysarthria in individuals who were exposed *in utero* and sensory and motor impairment in exposed adults. Chronic, low-dose prenatal methylmercury exposure from maternal consumption of fish has been associated with more subtle endpoints of neurotoxicity in children. Results from animal studies also show effects on cognitive, motor, and sensory functions. The following section focuses on studies reporting neurotoxicity as an endpoint for methylmercury exposure.

3.2 NEUROTOXICITY

3.2.1 Human Studies

3.2.1.1 Minamata and Niigata, Japan

Minamata Bay, Japan

The first documented widespread human methylmercury poisoning occurred in Minamata, Japan, between 1953 and 1960. Over time the source of the poisoning was traced to consumption of contaminated fish and seafood from Minamata Bay. An industrial plant was found to have discharged waste containing mercury directly into the waters of the bay. The initial cases of what was later called Minamata disease were two young women with what appeared to be encephalitis. Public awareness of the situation grew after the sudden deaths of cats in the surrounding area. Cats were brought into Minamata in February 1957 to study the possible health impact of environmental exposure to methylmercury. Within 32 to 65 days after arrival, all developed similar symptoms (e.g., excessive salivation, violent rotational movements, inability to walk in a straight line, and collapsing death or voluntarily jumping into the sea to drown) (Harada, 1995). This episode revealed the potential neurotoxic effects on humans exposed to methylmercury.

Adult Minamata Disease

Officially, approximately 2,200 persons have Minamata disease. Many other cases of the disease have either not been reported or were misdiagnosed. Many had eaten contaminated fish and shellfish for quite some time before the symptoms appeared (Iwata et al., 1975). In human patients, the early stage of Minamata disease brought gross disturbance of the central nervous system, which affected approximately 88 people living in the area around Minamata Bay. Of those 88 people, 12 died within 100 days, while the others had permanent disability. Among those with permanent disability, symptoms included appallic symptoms and idiotic disorders, with nervous symptoms resulting from widespread disturbance of brain cortices. In those with advanced illnesses from moderate poisoning, symptoms included tremor, disturbance of sensation, severe generalized ataxia, dysarthria, concentric constriction of the visual fields, and difficultly in hearing (Takeuchi et al., 1975).

The most common clinical signs observed in adults were paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing, and difficulty in walking. Examination of the brains of severely affected patients who died revealed marked atrophy of the brain (55% normal volume and weight), with lesions in the cerebral cortex and cerebellar cortex, and changes in the nerve fibers, cystic cavities, and spongy foci (Harada, 1995). Microscopically, entire regions of the brain were devoid of neurons, granular cells in the cerebellum, Golgi cells, and Purkinje cells. In addition to effects on the brain, methylmercury is known to have direct effects on the visual field. Korogi et al. (1997) presented results from a study on the comparison of magnetic resonance imaging findings of the striate cortex with visual field deficits in patients with Minamata disease. Results from this study indicated that the central 10° and 15° of vision represent 20% and 30% of the surface area of the striate cortex, respectively. The central portion of the visual fields occupied the posterior area as well as a greater proportion of the striate cortex. The visual field deficits in patients with Minamata disease correlated well with the magnetic resonance findings of the striate cortex. In severe cases of Minamata disease, the visual fields are identical with bilateral homonymous hemianopsia, with sparing of central vision (Korogi et al., 1997).

Delayed Onset-Type Minamata Disease

Mercury content in the hair and blood samples of Minamata patients was not analyzed until 1959. This was due in large part to the latency of the disease; the Minamata incident had apparently continued for such a protracted period that symptoms were delayed in appearing. In some cases, symptoms appeared more than 5 years after methylmercury intake ceased. Symptoms of delayed Minamata also were complicated by other diseases or aging. In the case of maternal exposure, symptoms usually did not appear until 5 to 8 years after the birth of the child. At this time, hair samples from mothers ranged from 1.82 to 191 ppm, while that of their offspring (congenital patients) ranged from 5.25 to 110 ppm (Harada, 1995).

Congenital Minamata Disease

Awareness of the developing fetus as a sensitive subpopulation came to light when a number of children were born with congenital cerebral palsy. These patients experienced symptoms such as mental retardation, primitive reflex, cerebellar ataxia, disturbances in physical development and nutrition, dysarthria, deformity of the limbs, hyperkinesia, hypersalivation, paroxysmal symptoms, strabismus, and pyramidal symptoms. Pathological findings of congenital Minamata disease patients include general atrophy and hypoplasia of the brain cortex and abnormality of the cytoarchitecture, remaining matrix

cells, hypoplasia of the corpus callosum, intramedullary preservation of the nerve cells, and dysmyelination of the pyramidal tract. In the cerebellum, hypoplasia of the granular cell layer and other layers as well as degeneration of granular cells were observed (Harada, 1995).

In a small fishing village called Yudo, 7 cases of cerebral palsy and 10 cases of infantile Minamata disease were found in a total of 50 households. Between 1955 and 1958, there were 188 births in the small fishing villages of Yudo, Tsukinowa, and Modo, with a 9.0% incidence of cerebral palsy, while the overall national incidence ranged from 0.2% to 2.3% (Harada, 1995).

Extensive investigations of congenital Minamata disease were undertaken and 20 cases that occurred over a 4-year period were documented. The exact number of congenital Minamata disease patients is not known, as some undiagnosed patients were already deceased. At present, 64 cases have been confirmed as congenital Minamata disease. In all instances congenital cases showed a higher incidence of symptoms than did the cases where exposure occurred as an adult. The congenital patients are unable to perform ordinary functions of living (Harada, 1995).

From 1950 to 1969, a total of 151 umbilical cords were collected from residents of the Minamata area. Included in this pool were 25 patients with congenital Minamata disease. Levels of methylmercury in the umbilical cords ranged from 0.35 ppm in 1952 to 0.96 ppm in 1955. The methylmercury levels in the cords from patients with congenital Minamata disease showed higher values than the cords of patients who had Minamata disease (0.72 ppm), mental retardation (0.74 ppm), other diseases (0.22 ppm), and no symptoms (0.28 ppm) (Harada et al., 1999).

Kinjo et al. (1993)

A case-control study examined the relationship between health complaints of patients with Minamata disease and exposure to methylmercury. A total of 1,144 Minamata disease patients older than 40 years of age were surveyed. A control group was also established; this group included nonexposed people living in neighboring towns, matched by age and sex. A questionnaire was used to obtain information on subjective complaints and activities of daily living (ADL). Results from analysis of the data indicated that Minamata disease patients had significantly higher rates of all complaints than did controls. Subjective complaints of Minamata disease patients, overall, were more prevalent than in controls. The results remained unchanged with age when the subjective complaints were categorized into two groups: those where frequency increased with age and those related to sensory disturbance. The

authors noted that the reason for the high prevalence rate of sensory disturbance among current Minamata disease patients is unclear. The data from the ADL questionnaire, when analyzed, were used to estimate functional capability in the elderly. Results indicate that ADL was significantly lower for Minamata disease patients aged 60 and over in comparison with controls. The authors conclude that ADL disability in Minamata disease patients is accelerated by aging. Overall, the prevalence of deficits was relatively greater in cases compared with controls as a function of increasing age.

Harada et al. (1998)

In 1995, Harada et al. (1998) measured mercury concentration in hair samples from 191 fishermen and family members living in mercury-polluted areas in the Minamata region of Japan. The study participants fished for a living and had previously consumed methylmercury-contaminated fish and shellfish caught in this region. Estimates of fish consumption were not provided. The study population comprised 83 men and 108 women who ranged in age from 32 to 82 years. Data on subjective symptoms and lifestyle factors were collected by questionnaire. In addition, each participant was administered relevant neurological tests (test details not provided) by a group of neurologists. Mercury concentrations in hair were less than 10 ppm in 185 out of 191 subjects. The mean concentrations were 5.0 ± 3.4 ppm and 2.1 ± 1.1 ppm for men and women, respectively. All six subjects with hair concentrations greater than 10 ppm were men. The mean concentration for men in the study was only slightly higher than the mean value of 4.6 ppm for normal nonexposed Japanese men. There appeared to be an upward trend in hair mercury concentration associated with increased frequency of fish consumption. Although the hair mercury concentrations approached what was considered normal ($\leq$10 ppm in hair samples), the study participants exhibited a high incidence of a variety of neurological conditions. More than 85% of subjects reported subjective symptoms including numbness, forgetfulness, pain in the extremities, focal cramps, headache, and motor disturbances. Clinical findings included sensory disturbance, ataxia, speech impediment, hearing impairment, constriction of visual fields, and tremor. "Stocking and glove" sensory disturbance (a hallmark of Minamata disease) occurred in 69% of the participants. A dose-response relationship between clinical symptoms and hair concentration was not evident, indicating that hair level data were of limited use for diagnosis of chronic Minamata disease.

Fukuda et al. (1999)

A study was completed in Kumamoto, Japan, near Minamata City, to evaluate the relationship between the number of neurological complaints from symptoms and methylmercury exposure. A total of

1,304 exposed adults living in a methylmercury-polluted area and 446 nonexposed age-matched adults, living in an area not known to be polluted with methylmercury, participated in an interview and questionnaire survey. The data from 64 participants of the survey were analyzed by comparison of prevalence, factor analysis, and cluster analysis. Results indicated that the exposed population had more neurological complaints in comparison with those not exposed. The factor analysis proposed four factors: arthritic, muscular, sensory, and nonspecific complaints. All four were higher in the exposed population in comparison with the nonexposed. The authors suggest that the increased neurological and nonspecific complaints may be due to past exposure to methylmercury.

Futatsuka et al. (2000)

A case-control study was conducted to estimate the role of various risk factors, including methylmercury exposure, for diseases such as liver disease, renal disease, and diabetes mellitus. The study population included 1,500 subjects over 40 years of age living in the town of Tsunagi since 1984. The town of Tsunagi was methylmercury polluted, with 36.9 diagnosed Minamata disease patients for every 1,000 population. Urine, blood, physical, and ultrasonographic examinations were administered to determine evidence of liver disease, renal disease, and diabetes mellitus. Personal interviews were conducted to collect information on risk factors and specific details on the complaints. Results from this study indicated that prevalence of disease, liver disease, renal disease, and diabetes mellitus was not higher in the methylmercury-polluted area compared with other areas in Japan. However, subjects in the polluted area had more complaints than those in the nonpolluted area. The authors concluded that past exposure to methylmercury may have influenced these results.

Niigata, Japan

From 1963 to 1965, patients with Minamata disease-like symptoms were reported in the basin of the Agano River in Niigata. Methylmercury, a residual product from acetoaldehyde synthesis, was released from a manure factory located 70 km up the river. Untreated wastewater from the factory drained into the Agano River, contaminating the fish and shellfish population. By 1973, 325 patients with Minamata disease were identified. This poisoning was later named "Niigata Minamata disease." Similar to the incident in Minamata, the symptoms progressed even after cessation of exposure. Numbness in the extremities and in the perioral area was the most frequently reported (Iwata et al., 1975). In the Niigata incident, the maternal hair mercury concentration immediately after giving birth to a congenital patient was 293 ppm. The maternal symptoms associated with this level of exposure were

mild, with sensory disturbances and other Minamata disease-related symptoms. The level of mercury exposure required to initiate the onset of Minamata disease was established at 50 ppm maternal mercury hair level. Because of the previous experience in Minamata with methylmercury poisoning, women with hair mercury levels above 50 ppm were advised not to become pregnant. As a consequence, there was only one case of congenital Minamata disease in the Niigata incident (Harada, 1995).

3.2.1.2 Iraq Outbreak

In fall 1971, 90,000 metric tons of methylmercury-treated seed grain were imported through the southern seaport of Basra, Iraq, and distributed freely throughout the countryside. Because the grain was delivered at planting time, residents of the area baked the grain into bread. There are no records on the size of the population who consumed grain treated with methylmercury fungicide. Nor are there reliable estimates of the number of people who ate methylmercury-treated grain and developed signs and symptoms but did not seek medical attention. It was not until late December 1971 that the first case of methylmercury poisoning was recorded. Within 2 months, 6,530 hospital admissions and 459 hospital deaths were recorded from methylmercury ingestion. Included in this exposed population were pregnant women (Bakir et al., 1973). Children exposed *in utero* manifested severe sensory impairments such as blindness and deafness, general paralysis, hyperactive reflexes, cerebral palsy, and impaired mental development (Amin-Zaki et al., 1974).

A study was conducted by Marsh et al. (1987) to investigate the relationship between methylmercury exposure, as measured by maternal hair concentrations during pregnancy, and associated adverse effects in offspring. A total of 81 mother-infant pairs participated; maternal hair mercury levels served as the index for prenatal exposure and were measured by x-ray fluorescent spectrometric analysis to range from 1 to 674 ppm. Clinical evaluations were conducted along with interviews with the mother about labor, delivery, any abnormalities at birth, size of the baby, early childhood development, and age at which infants achieved developmental milestones. These milestones included sitting without support, standing and walking unaided, and speaking two or three meaningful words. Developmental retardation was indicated by the child's inability to walk a few steps unsupported by 18 months of age or to speak two or three meaningful words by 24 months of age. Additional questions included any observations of involuntary movements, seizures, impaired vision or hearing, lack of coordination, and the mother's general impression of the child's physical and mental development. The interview was limited by the mothers' recall of the age of their children; moreover, this culture did not use Western calendars to record family events. The physical examination of the child included observation; head circumference

and body length measurements; cranial nerve signs; speech; limb tone, strength; deep tendon reflexes; plantar responses; coordination; dexterity; primitive reflexes; sensation; posture; and ability to sit, stand, walk, and run. Neurological examinations scored 0 to indicate normal functions and 3 to indicate definite abnormality. Unclear readings were denoted with points for borderline findings, whereas scores of 0-3 reflect no definite abnormality. The highest score in the most severely affected child was 11.

The impact of methylmercury on neurological function of infants exposed *in utero* during the Iraqi poisoning incident is described in a series of reports by Amin-Zaki et al. (1974, 1976, 1979, 1981), Marsh et al. (1980, 1981, 1987), and Seafood Safety (1991). The major symptoms observed in this epidemic closely resembled those recorded in Minamata, Japan. The predominant symptom noted in adults was paresthesia, and it usually occurred after a latent period of 16 to 38 days following initiation of exposure. Additional dose-dependent symptoms observed in the more severely affected individuals included ataxia, blurred vision, and constriction of the visual field leading to blindness in severe cases, slurred speech and hearing difficulties. Fatalities from methylmercury exposure usually resulted from failure of the central nervous system (Bakir et al., 1973). Of the 28 children with the highest exposures, 7 had seizures, whereas none of the 53 children with the lowest exposures experienced seizures. Maternal hair mercury levels for those seven children ranged between 78 and 674 ppm.

Results indicate that boys appeared to be more severely affected than girls. Statistically significant differences were apparent for regressions for boys and girls, where boys had the steeper slope to indicate increased severity in late walking and talking than girls.

Cox et al. (1989) performed an analysis of the Iraqi data to identify the threshold for adverse neurodevelopmental effects if one existed. A variety of statistical models such as logit, hockey-stick, and nonparametric kernel-smoothing methods were used in the attempt. Analyses were limited by the lack of data on the background prevalence of poor outcomes among Iraqi children. The authors estimated a population threshold of approximately 10 ppm for the outcomes investigated. The uncertainty associated with such an estimate, however, is highly dependent upon the assumed background prevalence of poor outcomes (e.g., motor retardation, neurological abnormality) (Cox et al., 1989). In another attempt at reanalyzing the data, Crump et al. (1995) reported that the estimate of the population threshold was highly dependent on the choice of the model and highly sensitive to the definition of abnormality. For example, delayed walking was heavily influenced by four cases of delayed walking among children with corresponding maternal hair mercury levels below 150 ppm. Crump et al. (1995) concluded that the statistical upper limit of the threshold could be as high as 255 ppm. Furthermore, their maximum

likelihood estimate of the threshold using a different parametric model was said by the authors to be virtually zero.

Cox et al. (1995) analyzed the Iraqi data on late walking in children exposed to methylmercury *in utero*. The results indicated that dose-response analyses based on late walking endpoints were unreliable because of four influential observations in the group of responders with hair mercury levels below 150 ppm. Based on visual interpretation of the plot of the data, the four observations are isolated from the remainder of the responders and would be expected to have considerable influence on the threshold estimate. No quantitative sensitivity analysis was performed to further investigate the effect of removing one or more of these data points. The authors point out that if the four data points were to represent background, the threshold for late walking would be greater than 100 ppm. This is, however, considered unlikely given that no responses were observed in the 37 individuals with lower levels of exposure.

3.2.1.3 Peru

A prospective study (Marsh et al., 1995) was conducted in Mancora, Peru, between 1981 and 1984 but not published until 1995. Mancora was selected as the study site based on a number of criteria, but mainly for its dependence on marine fish as a large source of dietary protein. A diet high in seafood was presumed to be associated with methylmercury exposure. Study participants consisted of 369 pregnant women and 194 of their children. Maternal hair samples were collected from the final group of 131 mother-infant pairs to analyze for methylmercury content. The geometric mean hair level was 7.05 ppm, with a range of 0.9 to 28.5 ppm. The peak maternal hair methylmercury levels during pregnancy ranged from 1.2 to 30 ppm, with a geometric mean of 8.3 ppm. Neurological examinations were administered to children. Frequencies were reported for tone decreased; tone increased; limb weakness; reflexes decreased; Babinski's sign, which is an indicator of a pyramidal-tract abnormality; primitive reflexes; and ataxia. This study identified no significant relationship between maternal hair methylmercury levels and measures of infant development or neurological signs. The authors suggested that marine fish may contain elements, such as selenium, that reduce the toxicity of methylmercury, thereby masking any neurological effects associated with methylmercury exposure.

3.2.1.4 Northern Quebec, Canada

A cross-sectional study of 234 Cree Indian children between the ages of 12 and 30 months on July 1, 1978, was conducted by McKeown-Eyssen et al. (1983). These children resided in four northern

Quebec communities known to have the highest levels of methylmercury exposures within Quebec. Maternal hair mercury level was the index to reflect prenatal exposure. Methylmercury levels of the hair were measured in alternate 1-cm segments, beginning with the scalp-end segment. The average maternal hair methylmercury concentration was 6 ppm, with only 6% of the samples exceeding 20 ppm. Physical and neurologic examinations were administered to the children, with the additional measures of special senses, cranial nerve function, sensory function, muscle tone, stretch reflexes, coordination, persistence of Babinski's response, and a summary of signs for the absence or presence of neurologic abnormality. At 4 years of age, four measures of the Denver Developmental Scale (gross and fine motor development, language development, and personal and social skills) were administered to assess the child's development. Associations between exposure and neurological outcome were analyzed by multiple regression analyses adjusted for alcohol and caffeine intake, tobacco use, age of mother, and multiparity.

No significant association between methylmercury exposure and neurological deficits was identified in girls. Abnormality of tendon reflexes was evidenced in 11.4% of the boys and 12.2% of the girls, but was only significantly associated with maternal hair mercury in boys. The prevalence of abnormality of muscle tone or reflexes was found to increase seven times with each increase of 10 ppm of the prenatal exposure index. However, the authors caution the interpretation of the results on boys because the abnormality of muscle tone or reflexes tended to consist of isolated abnormalities of mild severity that are of doubtful clinical importance. In addition, there was no dose-response relationship.

3.2.1.5 Seychelles Islands

The Seychelles Child Development Study (SCDS) was initiated in 1981 to examine the effects of low-dose fetal exposure to methylmercury from maternal consumption of fish. The SCDS was planned and conducted in two separate stages. The preliminary cross-sectional stage of the study sought to provide additional detail and guidance on how to design the main study. The main study, started in 1989, was a double-blind, prospective, longitudinally designed study that followed a cohort of infant-mother pairs from 6 months to 66 months postgestation.

Demographics

The Seychelles Islands is a Westernized archipelago in the middle of the Indian Ocean, more than 1,500 kilometers from the eastern coast of mainland Africa. The Seychellois population is of African and European origin with some minority groups from India and China. English, French, and Creole are

the three official national languages, with Creole being the most popular language at home. A majority (~85%) of the population consume a high amount of marine fish on a daily basis. In general, the Seychellois population is considered quite healthy, with easy access to good health care and education (Marsh et al., 1995).

Cross-Sectional Pilot Study (Myers et al., 1995b,c)

From 1987 to 1988, a cohort of 789 mother-infant pairs was selected after exclusion criteria were exercised. The fetal exposure index used was maternal hair total mercury. The levels ranged from 0.59 to 36.4 ppm, while the median level in this study was 6.6 ppm total mercury. The Denver Developmental Screening Test-Revised (DDST-R) was administered and a medical and neurological examination was performed for each child between 5 and 109 weeks of age. Covariates were selected for statistical analysis because of their potential to bias the assessment of the association between maternal mercury and developmental outcomes. These covariates included gender, birth weight, Apgar score, age at testing, and medical history. Mother's age, use of alcohol and tobacco, and medical history also were used. When DDST-R scores of questionable and abnormal results were grouped, mercury effects were seen and were more pronounced in boys and declined as age of testing increased. In general, males had higher response rates on the DDST-R than females, independent of mercury level. No association, however, was observed between mercury exposure and overall neurological examination results. The authors cautioned the interpretation of the results because the developmental association with fetal mercury exposure disappeared when DDST-R scores of "questionable" were treated in the standard manner as passes.

A subset (217 children) of the children from the pilot study cohort (Myers et al., 1995a) was tested at 66 months of age with the same battery of tests as planned for the main study at similar age. Maternal hair mercury levels during pregnancy ranged from 1.0 to 36.4 ppm, while the median level was 7.1 ppm. Nine endpoints were evaluated in this second evaluation: the McCarthy Scales of Children's Abilities that yield the general cognitive index (GCI), perceptual performance, memory, and motor ability; the Preschool Language Scale that yields total language score and subscores for verbal ability and auditory comprehension; and the letter-word identification and applied problems subscales of the Woodcock-Johnson Tests of Achievement. The association between maternal hair mercury concentration and outcome was assessed by multiple regression analysis. Prenatal mercury exposure correlated with outcomes at 66 months on the McCarthy GCI and perceptual performance subscale and with total language and auditory comprehension scores. After removing outliers and influential points, however,

mercury effects were no longer significant except for the Preschool Language Scale auditory comprehension subscale.

Prospective Longitudinal Main Study

A double-blinded, prospective longitudinal study was initiated with a new cohort of 740 mother-infant pairs that were selected between 1989 and 1990. These participants resided on the island of Mahe, which is one of the largest islands in the archipelago of the Seychelles where 90% of all Seychellois citizens live. Maternal hair mercury level was used as the marker of fetal mercury exposure. The levels ranged from 0.5 ppm to 26.7 ppm, with a median of 5.9 ppm. The cohort was followed from ages 6.5 months to 66 months, with evaluations occurring uniformly at four critical periods (6.5, 19, 29, and 66 months of age) (Myers et al., 1995). Tests of 7-year-old children have also been done, but results are not yet published. Age-appropriate tests were administered at the time points indicated in Table 3-1.

6-Month Evaluation (Myers et al., 1995c)

At 6 months of age, all children were administered a standardized test of visual recognition memory (Fagan Infantest); a standardized screening test to measure personal-social, fine motor adaptive, language, and gross motor development (DDST-R); and a general medical and neurological examination. Covariates of this main study included those evaluated in the pilot study, with the addition of birth order, gestational age of the child, primary caregiver intelligence, maternal and paternal educational levels, history of breastfeeding, language spoken at home, and family income. Medical conditions related to poor neurodevelopmental outcomes were also included as covariates in the statistical analysis. The study results indicate no association at 6 months of age with DDST-R, neurological examination, and Fagan Infantest. However, males had lower scores on both tests than females.

19- and 29-Month Evaluations (Davidson et al., 1995)

At 19 months of age, children were evaluated with the Bayley Scales of Infant Development (BSID), while the primary caregiver was administered the Raven Standard Progressive Matrices. The cohort was evaluated again at 29 months. Infant intelligence was measured by BSID Mental and Psychomotor Scales. To measure adaptive behaviors, a modified version of the BSID Infant Behavior Record was completed at 29 months. Between the ages of 42 and 56 months, children were administered

Table 3-1. Developmental domains evaluated and tests applied in the Seychelles Islands Child Development Main Study

Developmental Domain	Age of Child (months)			
	6.5	19	29	66
Marsh et al. (1995)				
Global-cognitive	DDST-R	BSID MDI	BSID MDI	MSCA GCI
Visual-perceptive	—	Kohen-Raz	Kohen-Raz	Bender-Gestalt MSCA Perceptual
Speech-language	DDST-R	—	—	MSCA Verbal PLS Total Language Aud. Comprehension Verbal Ability
Memory	Fagan Infantest	—	—	MSCA Memory
Visual attention	Fagan Infantest	—	—	—
Neuromotor exam	Neurological DDST-R	BSID PDI	BSID PDI	Bender-Gestalt MSCA Motor
Behavioral	DDST-R	—	BSID IBR	CBCL
Learning-achievement	—	—	—	Woodcock-Johnson
Auditory response	—	—	—	Audiometry Tympanometry
Davidson et al. (1998)				
Global-cognitive	—	—	—	MSCA GCI
Visual-perceptive	—	—	—	Bender-Gestalt
Speech-language	—	—	—	PLS Total Score
Behavioral	—	—	—	CBCL
Learning-achievement	—	—	—	Woodcock-Johnson Letter and Word Recognition, Applied Problems

Symbols and Abbreviations: — = No test administered; BSID = Bailey Scales of Infant Development; IBR = Infant Behavior Record; MDI = Mental Developmental Index; PDI = Psychomotor Developmental Index; CBCL = Child Behavior Checklist; DDST-R = Denver Developmental Screening Test - Revised; GCI = General Cognitive Index; MSCA = McCarthy Scales of Children's Abilities; PLS = Preschool Language Scale.
Source: Marsh et al. (1995); Davidson et al. (1998).

the Pre-School Caldwell-Bradley Home Observation for Measurement of the Environment (HOME). Hair samples were collected from all children at both 19 and 29 months of age for analysis of total mercury concentration to determine postnatal exposure. The median maternal hair mercury concentration during pregnancy for the 738 mother-infant pairs in the cohort at 19 months was 5.8 ppm. Twenty-two percent of the children at 19 months had child hair mercury levels ≥ 10 ppm (Myers et al., 1997). The same covariates and modeling strategy were used as in the primary analysis. No effects of mercury were detected on the BSID scores at either age. Results of this study indicate that one functional behavior—the examiner's subjective rating of the child's test session activity level—was related to maternal hair mercury levels in the mothers of male children: activity level decreased as maternal hair mercury level increased. Independent of mercury exposure; activity level was rated higher in males. Authors of this study conclude that these two results suggest that prenatal exposure to mercury may lower activity level in males. This result should be interpreted with caution as it is not yet clear whether the lower activity in males is a direct result of increased mercury exposure.

19-Month Evaluation of Walking and Talking (Myers et al., 1997)

The 19-month cohort was selected for evaluations of two developmental milestones. Data for age of first walking (n = 720) and talking (n = 680) were obtained from the primary caregiver of each child. Age at walking was defined as the age when the child was able to walk without support, while age at talking was defined as the age the child first said words other than "mama" and "dada." The mean age for walking was 10.7 months for girls and 10.6 months for boys, while for talking it was 10.5 months for girls and 11.0 months for boys. Multiple regression analysis was used to assess the relationships between each developmental milestone, maternal hair mercury levels, and covariates. Covariates evaluated are the same as those included in the study reported by Davidson et al. (1995) described in the previous paragraph. In this study, there was a marginally significant relationship between prenatal mercury exposure from eating fish and the age at which males started to walk, but this depended on four statistical outliers. No association between prenatal mercury exposure and either the age at which females started to walk or either gender started to talk was found.

Semiparametric Modeling of the 19-Month Data (Axtell et al., 1998)

In addition to the multiple regression analysis used in the prospective longitudinal main study of the SCDS, a semiparametric generalized additive model was used to identify nonlinearities in the relationship between prenatal methylmercury exposure and developmental milestone achievements. The

specific milestones evaluated in the main SCDS cohort at 19 months of age (n = 738 children) were age that children walked and said words. Walking was defined as the number of steps without support and talking was any word except "mama" or "dada." Maternal hair total mercury was used as an index of fetal exposure. No significant nonlinear relationships with mercury were identified in any of the models for age at talking; this implies that the original linear regression models were appropriate for this analysis. A General Additive Model analysis indicated that the relationship between maternal hair mercury level and age at walking may not be linear. Walking appeared at a later age as exposure increased in the range from 0 to 7 ppm. Walking appeared slightly earlier with increasing mercury levels above 7 ppm. However, there was no evidence from any models that higher levels of mercury exposure resulted in further delays in walking. There is no biological or developmental hypothesis to explain the increase in age of walking at lower levels and not at higher levels.

66-Month Evaluation (Davidson et al., 1998)

An evaluation was conducted on 711 mother-child pairs at 66 months of age. At this age, six neurobehavioral tests were administered: McCarthy Scales of Children's Abilities, the Preschool Language Scale, the Woodcock-Johnson Applied Problems and Letter and Word Recognition Tests of Achievement, the Bender Gestalt Test, and the Child Behavior Checklist (CBCL). Maternal hair mercury and child hair mercury were measured. Mercury exposure was assessed by total mercury in segments of maternal hair representing growth during pregnancy. The mean maternal hair total mercury level was 6.8 ppm while the mean child hair total mercury level at age 66 months was 6.5 ppm. The covariates evaluated include all those included in the previous study period, in addition to hearing status of the child and Hollingshead socioeconomic status of the family. Two multiple linear regression analyses were performed for each of the six primary measures. Secondary analyses tested the hypothesis that associations between developmental outcomes and total mercury exposure might be nonlinear. Four of the six measures (all except for Bender Gestalt and Woodcock-Johnson Applied Problems Tests of Achievement) showed better scores in the highest methylmercury groups compared with lower groups for both prenatal and postnatal exposure. For both prenatal and postnatal methylmercury exposure, no adverse developmental effects were reported for toddlers. Postnatal exposure at 66 months, however, was associated with a small but statistically significant increase on several developmental outcomes even though there is no reason to suppose that such effects are associated with exposure to methylmercury. There are studies, however, that indicate the methylmercury levels in the infant were surrogate for the length of breastfeeding, which is reported to have a positive association with developmental outcomes (Grandjean et al. 1992).

New Analysis—CBCL Main Cohort 66 Months (Myers et al., 2000)

No effect of mercury was identified on the Child Behavior Check List (CBCL) at 66 months of age in the main cohort of the Seychelles study as determined by the total T score (Davidson et al., 1998). The CBCL is a report inventory scored by the caregiver that assesses eight domains: withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior. An analysis of these subscales was performed on the 711 children assessed on this test (Myers et al., 2000). No effect of mercury was identified on individual subscales.

New Analysis—Main Cohort 66 Months (Axtell et al., 2000; Palumbo et al., 2000)

The investigators performed additional analyses of the 66-month data to evaluate the possibility of nonlinear relationships associated with mercury exposure (Axtell et al., 2000). Endpoints included the six primary variables analyzed previously: McCarthy GCI, Preschool Language Scale (PLS), Wodcock-Johnson Applied Problems, Woodcock-Johnson Letter/Word Recognition, Bender copying errors, and CBCL total T score. Generalized additive models, which make no assumptions about the relationship between exposure and test score, were used. Maternal hair levels during pregnancy were used as a measure of prenatal exposure and child's hair mercury at 66 months was used for postnatal exposure. Nonlinearities were identified between prenatal exposure and PLS and CBCL, and between postnatal exposure and McCarthy GCI. For the PLS the trend involved a decrement of 0.8 points (poorer performance) from 0-10 ppm and an increase of 1.3 points above 10 ppm. For the CBCL there was an increase (representing a poorer score) between 0 and 15 ppm and a decrease above 10 ppm. The GCI increased (improved) by 1.8 points through 10 ppm mercury in the child's hair and declined by 3.1 above 10 ppm. Although these results are difficult to interpret, they provide limited evidence of an adverse effect of mercury exposure below 10 ppm maternal hair on two measures, and a somewhat greater association of adverse effects with child's hair mercury above 10 ppm on the GCI. As pointed out by the authors, there are fewer data points above 10 ppm (this is especially true for child's hair mercury), and therefore trends above this level are estimated less precisely.

The investigators in the Seychelles study further examined by multiple linear regression the results of the McCarthy GCI administered at 66 months (Palumbo et al., 2000). They analyzed the standard MSCA subscales and also constructed subscales to approximate the domains of cognitive functioning assessed in the Faroe Islands study: attention, executive function, expressive language, receptive language, nonverbal memory, visuospatial ability, visuomotor ability, and gross motor ability. They

found a positive association between child's hair mercury at 66 months and the standard memory subscale, with no other associations identified. As with all previous analyses of these variables, the raw scores were converted to "normative" scores. As pointed out by an OSTP panel (NIEHS 1998, Section 3.5 of the Confounders and Variables Section), the applicability of U.S. norms to this population is unclear, and the use of standardized scores may decrease sensitivity by collapsing different raw scores to one standard score.

Pilot Cohort Analysis at 108 Months (Davidson et al., 2000)

Further evaluation was performed on a portion of the Seychelles pilot cohort at 108 months of age (Davidson et al., 2000). Eighty-seven children were tested on five subtests of the WISC-III (Information, Block Design, Vocabulary, Digit Span, and Coding), California Verbal Learning Test (CVLT), Boston Naming Test (BNT), Beery-Buktenica Development Test of Visual Motor Integration (VMI) (copying geometric figures), Finger Tapping, grooved pegboard, Trailmaking (tracing the correct route through a form with a pencil), and the design memory subtest of the Wide Range Assessment of Memory and Learning (WRAML) (drawing each of four geometric designs from memory). Performance on BNT, VMI, and grooved pegboard showed a positive association (better performance) related to mercury exposure in males, with no effects identified in females. There were trends toward poorer performance related to mercury exposure for grooved pegboard in females ($p = 0.07$) as well as marginal p values on the full model that were not further analyzed (Finger Tapping, digit span). The investigators did not report power calculations, but with such a small number of subjects the power was probably quite low, so these largely negative results need to be interpreted with caution.

Benchmark Analysis (Crump et al., 2000)

A benchmark analysis (Crump et al., 2000) was conducted on data from the SCDS, with the goal of providing an alternative basis for deriving an appropriate human exposure level for methylmercury. The data modeled included responses from the neurological test batteries conducted at 6.5, 19, 29, and 66 months of age. In addition, data for developmental milestones (age first walked and age first talked) were analyzed. Maternal hair mercury concentrations measured in this study ranged from 0.5 to 26.7 ppm and averaged 6.8 ppm.

Most of the measured endpoints in the SCDS were recorded as continuous responses, and the *k*-power model, the Weibull model, and the logistics models for continuous data were applied. Test scores

below a predetermined value, $P_0 = 0.05$**,** were considered abnormal. For this analysis, the BMR was defined as 10% (BMR = 0.1). (For a description of modeling terms see Section 4.3).

In cases where responses were recorded as quantal responses (abnormal/normal), the data were modeled using the Weibull dose-response model for quantal data. Quantal responses reported in children in the Seychelles study included deep tendon reflexes, limb tone, overall neurological responses, and psychomotor index. In addition, each continuous response was converted to a quantal response by considering a response abnormal if it was more than 2 standard deviations away (in the adverse direction) from the mean response of the entire cohort, and then analyzed using the Weibull model. In these analyses, the BMD was defined in the same way as in the analyses of the continuous response.

The analyses of continuous response were conducted without covariates. Analyses with P_0 specified were conducted using both an expanded set and a reduced set of covariates for the children: sex, birth weight, birth order, whether or not the child was breastfed, medical history, maternal age, maternal smoking and alcohol use during pregnancy, maternal medical history, language spoken in home, score from home visit, Raven group (caregiver's intelligence quotient), maternal and paternal education level, family income, gestational age, Hollingshead socioeconomic scale, auditory scores, and the child's mercury level. Covariates were not included in the analyses of quantal responses or in the analyses of continuous responses in which x_0 was specified.

Parameter estimates were obtained using the maximum likelihood method, and statistical confidence bounds were computed by the profile likelihood method. The BMDL was defined conventionally as the 95% statistical lower confidence bound on the BMD. Results indicated that the most reliable analyses were represented by 144 calculated lower statistical bounds on the BMD (BMDL, or the lower statistical bound on maternal mercury hair level corresponding to an increase of 0.1 in the probability of an adverse response) derived from the modeling of continuous responses.

The results of BMD modeling are shown in Table 3-2. The average value of the BMDL in these 144 analyses was 25 ppm mercury in maternal hair, with a range of 19 to 30 ppm. With the exception of the linear model, which produced larger BMDLs, the dose-response models applied to continuous end points all produced comparable BMDLs.

Table 3-2. BMDL values (expressed as ppm mercury in maternal hair) for neurological responses and developmental milestones from the Seychelles Child Development Study

Endpoint	Model						
	Weibull				*K*-Power		
	P_0[a]		χ_0[b]	Quantal	P_0[a]		χ_0[b]
	None	Exp.[c]	None	None	None	Exp.	None
6.5 Months							
Deep tendon reflexes	—	—	—	22.8	—	—	—
Limb tone	—	—	—	20.9	—	—	—
Overall neurological	—	—	—	15.8	—	—	—
Fagan visual recognition memory	26.0	26.0	27.4	19.7	26.0	26.0	26.9
Fagan attention	25.7	25.9	27.0	23.7	25.5	25.6	26.4
19 Months							
Mental development index	23.7	23.4	26.0	22.6	24.3	24.1	25.6
Psychomotor index	—	—	—	22.3	—	—	—
29 Months							
Mental development index	24.1	24.4	25.7	21.9	24.0	24.2	24.8
Psychomotor index	—	—	—	22.5	—	—	—
66 Months							
Bender gestalt errors	26.9	26.7	28.5	22.7	26.7	26.7	27.5
Child behavior checklist total	27.2	27.2	29.0	19.4	20.0	26.9	27.8
McCarthy general cognitive index	24.4	24.2	26.5	22.7	24.7	24.6	25.9
Preschool language total score	25.2	25.1	26.8	22.7	24.7	24.7	25.5
Woodcock-Johnson							
Applied problems	23.1	23.5	25.3	22.7	23.9	24.3	25.5
Letter-word recognition	23.7	23.7	25.3	22.7	23.8	23.9	24.7
Developmental milestones							
Age first walked unassisted	24.9	24.0	25.9	22.7	24.4	23.2	26.8
Age first talked	24.6	23.5	25.9	20.3	25.0	24.1	25.9

[a] Abnormal defined as a response >2 standard deviations in adverse direction from mean response of entire cohort.
[b] Abnormal defined so that 5% of responses are abnormal ($p_0 = 0.05$).
[c] Exp. denotes use of an expanded range of covariates.

Source: Crump et al., 2000.

3.2.1.6 New Zealand

A study was conducted in the northern New Zealand islands to study the effects of prenatal methylmercury exposure on children exposed *in utero* from maternal fish consumption. Between 1982 and 1983, 11,000 mother-infant pairs were requested to submit hair samples and fill out a detailed diet questionnaire. Of those 11,000 pairs approximately 1,000 of these mothers had consumed fish more than three times per week for the 9 months of pregnancy. Seventy-three had hair mercury levels above 6 ppm, with the highest level being 86 mg/kg. This study was conducted in two stages.

Preliminary Tests at Age 4 (Kjellstrom et al., 1986)

From the 73 mothers with high mercury exposure (> 6 ppm) during pregnancy, a total of 31 matched pairs were selected to participate in a study on the effects of prenatal methylmercury exposure on children exposed *in utero* from maternal consumption of fish. A reference child matched for mother's ethnic group, age, and child's birthplace and birth date was located for each child selected from the high-fish-consumption group. Mercury exposure during gestation was determined from maternal hair analysis. The average hair concentrations for high-exposure mothers and the reference group were 8.8 ppm and 1.9 ppm, respectively. At 4 years of age, the children were tested using the DDST. Standardized vision tests and sensory tests were also performed to measure development of these components of the nervous system. The prevalence for developmental delay in children was 50% for progeny of high-mercury mothers and 17% for progeny of mothers of the control group. These results were statistically significant. Analysis of the DDST results by sector showed that developmental delays were most commonly noted in the fine motor and language sectors, but the differences between the experimental and control groups were not significant. The authors concluded that children born to mothers with mean hair mercury levels above 6 ppm have twice the risk of delayed development, as tested by the DDST, in comparison with the control group.

Psychological Tests at Age 6-7 (Kjellstrom et al., 1989)

In 1985 when the children were 6 to7 years of age, a follow-up study was conducted. In this study, 61 of the 74 high-exposure children were compared with three control groups with lower prenatal mercury exposure. Average maternal hair mercury concentrations in the control groups were 3 to 6 ppm and 0 to 3 ppm, respectively. The high-exposure group, with maternal hair mercury levels ranging from 6 to 86 ppm, was matched with controls for maternal ethnic group, age, smoking habits, residence, and sex

of the child. Each child was tested with a battery of 26 scholastic, psychological, and behavioral tests, which included Test of Language Development (TOLD), the Wechsler Intelligence Scale for Children (WISC), and McCarthy Scale of Children's Abilities as described in Table 3-3. Confounding factors such as language used at home, maternal and paternal occupation, maternal alcohol consumption, and number of children in the household were controlled using linear multiple regression analysis.

Table 3-3. Developmental domains evaluated and tests applied in studies of New Zealand children with prenatal exposure to mercury from fish

Developmental Domain	Age of Child (years)	
	4	6
General cognitive	—	MSCA general WISC-R Performance IQ, Total IQ
Visual-perceptual	Sheridan-Gardiner Letter Matching test Miniature Toy Test	MSCA perceptual
Speech-language	DDST	TOLD Spoken Language Quotient MSCA Verbal WISC-R Verbal Peabody Picture Vocabulary Test (1981)
Memory	—	MSCA memory
Motor	DDST	MSCA motoric
Learning-achievement	—	Clay Diagnostic Survey Concepts, Letter Test, and Word Test MSCA quantitative Burt Word Recognition Test Key Math Diagnostic Arithmetic Test
Personal-social	DDST	Everts Behaviour Rating Scale

Symbols and Abbreviations: — = No test administered; DDST = Denver Developmental Screening Test; MSCA = McCarthy Scales of Children's Abilities; TOLD = Test of Language Development; WISC-R = Wechsler Intelligence Scale for Children - Revised.
Source: Kjellström et al., 1986; 1989.

An average hair mercury level of 13 to 15 ppm during pregnancy was consistently associated with decreased test performance. Results of the psychological test variables were influenced by ethnic background and social class. After controlling for confounding factors and eliminating outliers, the association between prenatal methylmercury exposure and decreased performance in psychological tests remained unchanged. The children who had the poorest performance in the WISC IQ test at age 6 also had a high prevalence of abnormal or questionable DDST scores at age 4, indicating that the effects evidenced in this follow-up study confirm those found in the preliminary study at age 4. The authors

conclude that effects of methylmercury leading to developmental delays may later lead to deficits in psychological tests.

Benchmark Modeling of the 1985 Data (Crump et al., 1998)

Crump et al. (1998) performed a reanalysis and BMD modeling of the Kjellstrom et al. study results. Crump et al. used actual hair mercury levels as opposed to an indicator variable for mercury level in hair; additional confounding factors, such as parent's education and age at which the child was tested were also controlled for. They also and evaluated all 26 scholastic and psychological tests (illustrated in Table 3-4) administered to the 237 6 to 7-year old children. No significant associations between mercury exposure and children's test scores were identified. This finding, however, was highly influenced by one child whose mother's hair mercury level was 86 ppm, fourfold higher than observed for any other mother. When this outlier was omitted, scores on six tests were found to be significantly associated with maternal hair mercury concentrations: Clay reading test-concepts, Clay reading test-letter test, McCarthy-general cognitive test, McCarthy-perception, TOLD-grammar completion, and TOLD-grammar understanding. BMDs calculated from five tests (TOLD-spoken language quotient, WISC-performance IQ, WISC-full scale IQ, McCarthy perceptual, and McCarthy-motoric) ranged from 32 to 73 ppm and BMDL of 17 to 24 ppm, respectively. When the child with the highest maternal hair mercury was excluded, the BMDs ranged from 13 to 21 ppm with BMDLs spanning 7.4 to 10 ppm (Table 3-4).

Table 3-4. BMD and BMDL values (expressed as maternal hair mercury concentration, ppm) for neurobehavioral endpoints in New Zealand children evaluated at 6 to 7 years of age

Test	All New Zealand children		Child with highest maternal mercury concentration omitted	
	BMD[a]	BMDL[b]	BMD	BMDL
TOLD – spoken language	45	20	15	9.5
WISC – performance IQ	73	24	15	10
WISC–full-scale IQ	51	21	15	10
McCarthy–perception	32	17	13	7.4
McCarthy–motoric	55	21	21	9.8

[a] A background prevalence (P_0) of abnormal response of 5% and a benchmark response of 10% were used for these calculations.
[b] 95% lower confidence bound on BMD.
Abbreviations: TOLD = Test of Language Development; WISC = Wechsler Intelligence Scale for Children.
Source: Crump et al. (1998).

3.2.1.7 Faroe Islands

A large human prospective longitudinal study was conducted in the Faroe Islands to determine if increased methylmercury exposure is related to decreased neurobehavioral function. Before the prospective study, a pilot study was conducted to assess the magnitude of fetal mercury exposure in the Faroes. At 12 months of age, a follow-up evaluation was conducted and then a prospective study was initiated with children born at consecutive deliveries within a 22-month period at nearby hospitals.

Demographics

The Faroes is a group of 18 islands located in the North Atlantic between Scotland and Iceland. The Faroese population is homogenous with respect to cultural and socioeconomic factors. The culture is mainly Scandinavian, with a traditional stable family unit that has easy access to good health care, education, and social systems. Dietary deficiencies are virtually nonexistent, alcohol intake is low, rate of preterm delivery of low-birth-weight infants is also low, and rate of breastfeeding is high for at least 12 months (Budtz-Jorgensen et al., 2000). Seafood constitutes a major part of the average diet in fishing communities in the North Atlantic like the Faroe Islands (Grandjean et al., 1995). The major source of methylmercury exposure is pilot whale, which according to ancient tradition was hunted and distributed within the community (Grandjean et al., 1997). Other components of the Faroese diet include lamb, potatoes, dairy products, and foods imported from other countries (Steurwald et al., 2000).

Pilot Study (Grandjean et al., 1992)

A pilot study was conducted by Grandjean et al. (1992) to assess the magnitude of fetal mercury exposure in the small fishing village of Lorvik, Faroe Islands. Blood samples were collected from a group of 53 women of fertile age, between 20 and 50, identified through a municipal register. Between 1986 and 1987, 1,023 umbilical cord blood samples were also collected at consecutive deliveries at three local hospitals. Women had a median blood mercury level of 12.1 μg/L, with values that ranged from 2.6 to 50.1 μg/L. The median mercury concentration in cord blood for all 250 samples exceeded 40 μg/L, while 20 samples had levels higher than 100 μg/L. Hair samples had mercury content that exceeded 10 ppm, and five samples exceeded 25 ppm. In 34 hair samples the measured mercury levels exceeded 15 ppm. Mercury concentrations tended to be 20% to 65% higher in cord blood than in the venous blood of mothers. Highly increased mercury concentrations in maternal hair and umbilical cord blood were related to maternal consumption of pilot whale.

12-Month Evaluation (Grandjean et al., 1995b)

At 12 months of age, 583 children were selected for further evaluation. These children were followed for 1 year after birth. Three age-appropriate developmental milestones were evaluated: sitting, creeping, and standing. The age at which the child achieved a developmental milestone was not associated with indices of prenatal mercury exposure, either from cord blood (average of 174 μg/L) or maternal hair (approximately 15% of mothers had concentrations above 50 nmol/g). Infants who reached the milestone criteria early had significantly higher mercury concentrations in their hair at 12 months than those who did not. The child's hair mercury concentration was found to be highly correlated to the period of breastfeeding. Breast milk may transfer contaminants such as methylmercury, but it is also known to confer certain advantages such as maternal antibodies. The authors concluded that if methylmercury exposure from human milk had any adverse effect on milestone development in these 12 month-old infants, the effect was compensated for by advantages offered through breastfeeding.

Computer-Assisted Neurobehavioral Tests in 7-Year-Olds (Dahl et al., 1996)

In this study, 917 children were evaluated at 7 years of age. The study focused on computer-assisted neurobehavioral tests and whether or not they could serve as meaningful parameters of neurotoxicity; three Neurobehaviroal Evaluation System (NES) tests were administered with slight modifications. The NES tests were selected to assess motor speed (Finger Tapping [FT]), sustained attention (Continuous Performance Test [CPT]), and motor coordination (Hand-Eye Coordination [HEC] Test). The CPT was modified to use animal silhouettes as a stimuli instead of letters to accommodate those children who had not yet started school and were unfamiliar with the alphabet.

Finger Tapping was relatively easy for most children, but the HEC test was considered too difficult. Of the 914 children who completed the full HEC, 755 had fewer than 25% nonresponses. Decreased visual acuity, strabismus, use of eyeglasses, and contrast sensitivity were markedly associated with decreased performance, especially on the CPT. Boys and older children performed better than girls and younger children, but this was due to increased familiarity with computers and use of a joystick. The authors concluded that maternal hair mercury and cord blood mercury were clearly associated with NES results, especially in the FT and CPT tests.

Main Prospective Longitudinal Study of 7-Year-Olds (Grandjean et al., 1997)

The cohort consisted of 917 children at 7 years of age who survived from the original cohort established in the pilot study. Indices of prenatal exposure included cord blood and maternal hair, and the index for postnatal exposure was children's hair mercury. The geometric mean cord blood mercury concentration was 22.8 μg/L, and the concentration found in children's hair averaged 11.68 ppm. Detailed neurobehavioral and physical examinations and neuropsychological and neurophysiological testings were performed. The neuropsychological tests (Table 3-5) included NES FT Test, NES HEC Test, Tactual Performance Test, NES CPT, Wechsler Intelligence Scale for Children - Revised (WISC-R), WISC-R Similarities, WISC-R Block Designs, Bender Gestalt Test, California Verbal Learning Test-Children [CVLT]), Boston Naming Test (BNT), and Nonverbal Analogue Profile of Mood States. These tests were chosen for their sensitivities in detecting neuropathological abnormalities. The neurophysiological tests were chosen to exclude those with electrical stimulation or long measurement times. These tests include pattern reversal visual-evoked potentials with binocular full-field stimulation, brain stem auditory-evoked potentials (BAEP), and postural sway.

Fewer than 60% of the children completed three of the most difficult tests. The WISC-R Similarities Test, NES HEC Test, and Nonverbal Analoguous Profile of Mood States were found to be too difficult for many of the children to reveal the subtle neurotoxic effects associated with methylmercury. The geometric mean cord blood mercury concentration for the 85 children who failed or refused to take the mood test was 29.5 μg/L, compared with 22.3 μg/L in children who voluntarily completed it. Reciprocal motor coordination and simultaneous finger movement showed no relation to mercury exposure. In the finger opposition test, however, 465 children with geometric mean blood concentrations of 21.8 ug/L mercury performed optimally, whereas those with blood concentrations of 23.9 μg/L had questionable or deficient performances.

Mercury-related abnormalities were not identified in either the neurophysiological or clinical examination. However, in the neuropsychological testing, statistically significant mercury-related dysfunction was observed. This was most pronounced in the areas of language, attention, and memory, and to a lesser extent visuospatial and motor functions. After adjustment of covariates and exclusion of children with maternal hair mercury above 10 ppm, the association remained. This indicates effects of methylmercury at doses lower than that which result in 10 ppm maternal hair mercury. In the neurophysiological test, girls showed significantly shorter latencies of evoked potentials than boys in the electrophysiological tests. For the BAEP latencies, peak I at 40 Hz and 20 Hz was slightly delayed at

Table 3-5. Developmental domains evaluated and tests applied in studies of Faroese children at age 7 years

Developmental Domain	Test
Grandjean et al. (1997) - Main Prospective Study	
General cognitive	WISC-R Similarities
Visuospatial	WISC-R Block Designs Bender Motor Visual Gestalt Test
Attention	NES2 Continuous Performance WISC-R Digit Spans Forward
Speech-language	Boston Naming Test
Memory	California Verbal Learning Test
Motor	NES2 Finger Tapping NES2 Hand-Eye Coordination NES2 Tactual Performance
Personal-social	Nonverbal Analogue Profile of Mood States
Grandjean et al. (1998) - Nested Case Control Study	
General cognitive	WISC-R Similarities
Visuospatial	WISC-R Block Designs Bender Visual Motor Gestalt Test
Attention	NES2 Continuous Performance WISC-R Digit Spans Forward
Speech-language	Boston Naming
Memory	California Verbal Learning Test
Motor	NES2 Finger Tapping NES2 Hand-Eye Coordination
Personal-social	—

Symbols and Abbreviations: — = No test administered; NES2 = Neurobehavioral Evaluation System; WISC-R = Wechsler Intelligence Scale for Children - Revised.

Source: Grandjean et al., 1997, 1998.

increased prenatal mercury exposures and the delays for peaks III and V were statistically significant, but the interpeak latencies showed no associations with mercury. Body sway showed a slight negative association with mercury exposure in all four conditions: eyes open, no foam; eyes closed, no foam; eyes open with foam; and eyes closed with foam.

Four tests were selected for further analysis. Tests were chosen to reflect each of the following brain functions: motor function (Finger Tapping with preferred hand), attention (CPT reaction time), visuospatial performance (error score on the Bender Visual Motor Gestalt Test), language (Boston Naming Test after cues), and memory (long-delay recall on the California Verbal Learning Test). After

adjustment for covariates using the Peters-Belson method, children with scores in the lowest quartile were identified and distributed into quartile groups of mercury exposure (< 15, 15-30, 30-50, and > 50 μg/L). These results indicate that there is a statistically significant trend for the attention, language, and memory test with increasing prenatal mercury exposure (Grandjean et al., 1997).

Pilot whale blubber is also consumed by the Faroese population, and this could result in increased exposure to PCBs, a potential confounding factor. A subset (n = 436) of the cord tissue samples was evaluated for PCBs; inclusion of PCB exposure as a covariate in the regression analysis affected only the regression for the BNT. The authors conclude that results of the expanded data analysis do not suggest that the mercury effect can be explained by concomitant PCB exposure, or that PCB exposure enhances the mercury-associated effects.

Reevaluation of the Evoked Potentials in the Prospective Study (Murata et al., 1999a)

Significant associations with delays in evoked potential latencies and mercury exposure (Murata et al., 1999a) initiated the reanalyses of the data from the prospective longitudinal study. This analysis is limited to only children born during the first half of the cohort generation in 1993. Data from the second year were excluded because of shorter BAEP latencies and delayed latency on the visual-evoked potentials. Three sets of mercury exposure data were utilized in regression analyses: (1) mercury in cord blood (geometric mean of 23.0 μg/L, range of 3.3-351 μg/L), (2) mercury in maternal hair at parturition (geometric mean of 4.49 ppm, range of 0.9-39.1 ppm), and (3) mercury in the child's hair (geometric mean of 3.42 ppm, range of 0.04-26.4 ppm). The mercury concentration in maternal hair was a significant predictor for peak III latency and the I-III interval, where the child's own hair mercury concentration at the time of examination was not associated with these response variables. The cord blood concentration was, however, a significant predictor, supporting the notion that the latency delays are related to increased prenatal methylmercury exposure.

Nested Case-Control Study (Grandjean et al., 1998)

Following the evaluation of 7-year-olds in the prospective longitudinal study, the data were evaluated as a nested case-control study. From the original cohort of 1,022 established in the pilot study, the cases and controls were selected based on maternal hair mercury concentration. The case group of 112 children whose mothers had hair mercury concentrations of 10 to 20 ppm was matched to children with prenatal exposure below 3 ppm (control). Age, sex, time of examination, and maternal Raven score

were matching criteria. The median maternal hair mercury concentrations in the two groups were 1.8ppm for the control group and 12.5 ppm for the cases, a sevenfold difference. The median cord blood mercury concentrations for the control and cases were 11.9 and 59.0 μg/L, respectively. Neuropsychological tests evaluated were these: NES2 FT Test, NES2 HEC Test, NES2 CPT, WISC-R Similarities, WISC Block Designs, Bender Visual Motor Gestalt Test, CVLT, and BNT. The case group performed less satisfactorily than those in the control. On 6 of the 18 test outcomes, the inferior scores achieved by the case group were statistically significant. In particular, the case group showed a deficit on the Finger Tapping condition and the overall hand-eye coordination. Girls and boys scored differently on the Bender Gestalt Test, California Verbal Learning Test, all three Finger Tapping conditions, CPT reaction time, and the average hand-eye coordination score. No differences were reported between girls in the cases versus controls, but boys in the case group scored poorer in the Finger Tapping reaction time than the boys in the control group. The deficit in motor coordination, especially in Finger Tapping with both hands, was highly significant for boys only. The author noted that the findings of this matched case-control study are in accordance with regression analyses performed on all 900 children at the 7-year evaluation; methylmercury effects appear in the several domains of the brain, focusing on motor function, language, and memory.

Benchmark Modeling (Budtz-Jorgesen et al., 2000)

Benchmark modeling of the data from the Faroese children at 7 years of age was reported by Budtz-Jorgesen et al. (2000). The exposure was modeled both as mercury concentration in cord blood and in maternal hair. The number of children that completed neuropsychological tests varied between 837 and 901. One neuropsychological test was selected for evaluation of each of the five domains of brain function:

1. Motor speed (NES FT Test)
2. Attention: NES2 CPT
3. Visuospatial performance: Bender Visual Motor Gestalt Test
4. Language: BNT
5. Short-term memory: CVLT

For tests of motor function, language, and memory, a logarithmic dose-response model tended to show a better fit than a linear dose model using cord blood mercury concentration as the dose parameter. The default p_0 is 5%, which equates to the level (x_0) of abnormal test performance as defined by a probability

of 5% in the unexposed population. The Faroese cohort does not include an unexposed control group; thus the performance level for an unexposed child is obtained by fitting a dose-response curve to all data points, followed by extrapolating to zero exposure. Four different dose-response models were employed: *K* power, linear, square root, and logarithmic.

The results from this analysis indicate that BMDs and BMDLs vary substantially. Of the four models, the logarithmic dose-response model provided the best fit for some of the outcome variables that showed the closest association with the cord blood mercury concentration. The lowest BMDLs averaged approximately 5 µg/L cord blood, which is equivalent to approximately 1 ppm in maternal hair. Most BMDLs for hair mercury concentrations were higher. However, the results for a BMR of 5% are the same order of magnitude as the cord blood results at a BMR of 10%. The authors concluded that the results of the benchmark calculation are highly dependent on the assumed dose-response model. Results of this analysis are discussed further in the Risk Assessment chapter (Chapter 4). (For a description of modeling terms see Section 4.3).

Second Cohort (Steurwald et al., 2000)

During a period from 1994 to 1995, a second cohort of 182 singleton term births was generated from consecutive births at the National Hospital in Thorshavn, Faroe Islands (Steurwald et al., 2000). Maternal hair, serum, breast milk, and umbilical cord blood were analyzed for contaminants, while selenium, thyroid hormones, and fatty acids were measured in cord blood. In addition to methylmercury, PCBs were examined as a possible confounder in test outcome. At 2 weeks of age, infants were administered a neurological examination. Assessment of functional abilities, reflexes and responses, and stability of behavioral status during examination were completed with a score of optimal, questionable, or suboptimal performance. The Neurologic Optimality Score (NOS) was the number of items rated as optimal out of a total of 60. Results from this study indicate that prenatal exposure to methylmercury and PCBs increased from maternal intake of seafood. After adjustment for confounders, a tenfold increase of the cord blood mercury concentration was associated with a decreased NOS of 2.0. This effect corresponds to a decrease in gestational age of about 3 weeks. The authors conclude that prenatal exposure to methylmercury from contaminated seafood was associated with an increased risk of neurodevelopmental deficit. No evidence for a protective or beneficial effect with respect to neurological optimality score (the number of main items rated optimal out of 60) was observed for essential fatty acids or selenium.

3.2.1.8 Germany

Cross-Sectional Study (Altmann et al., 1998)

From a larger comparative environmental screening study, 384 children between the ages of 5 and 8 years were selected to participate in a smaller field experiment to investigate the effects of low-level lead and mercury exposure on the functions of the developing visual system. Blood lead levels and urinary excretion of lead and mercury were used as exposure indices. Neurophysiological and psychophysical measurements were administered to the children. Visual functions were assessed for neurophysiological measurements, while psychophysical measurements were assessed by visual-evoked potentials and contrast sensitivity. Linear regression analyses were used to analyze the possible relationship between exposure to lead and mercury and outcome variables. Adjustments were made for potential confounding factors such as parental education, birth weight, length of lactation, and premature birth.

After adjustment for potential confounding factors, contrast sensitivity values were significantly reduced with increasing urinary mercury levels; four of the ten contrast sensitivity values tested showed a statistically significant decrease with increasing urinary mercury. Very subtle changes in the visual system function were noted at very low levels of urinary mercury. However, no significant associations were found between urinary mercury output and any visually evoked potential outcome variables.

3.2.1.9 Nambija, Ecuador

Cross-Sectional Study on Neurosensory Dysfunction (Counter et al., 2000)

A cross-sectional study was conducted in the remote Andean settlement of Nambija, Ecuador, to investigate whether blood mercury levels are associated with auditory neurosensory dysfunction. Participants in this study included 36 children and 39 adults living in Nambija , an area known to have extensive gold-mining operations where mercury is used in the extraction process. Mercury exposure was measured in whole blood. The mean blood mercury level was 17.5 µg/L. A group of 34 subjects (15 children and 19 adults) from a non-gold-mining area were selected as the control group. Their mean blood mercury level was 3.0 µg/L. A neuro-otological examination was administered; a neurological examination of the cranial nerves was administered using standard procedures and an audiological test was administered to 21 children and 19 adults.

Of those examined, 45% of the group complained of headaches and/or memory loss, three cases involved severe neurological impairment and four cases involved middle ear pathology. A statistically significant relationship was identified between blood mercury level and hearing level in children at 3 kHz in the right ear only. Adults were not affected. BAEP responses showed a significant correlation between blood mercury and the I-III interpeak latency on the left side. The authors conclude that the findings of this study suggest that overall auditory sensory-neural function and neural conduction time at the brain stem level were generally unaffected by elevated blood mercury levels in either children or adults.

3.2.1.10 Amazonian Basin

The conditions in the Amazon—extremely high temperatures and humidity with seasonal fluctuation of water during rainy and dry seasons—are conducive for mercury methylation because of high quantities of suspended organic matter, high temperature, acidity, and redox potential. These elements influence the availability of fish as a food resource. In 1996, Lebel and colleagues published results from a small preliminary study on individuals from the Amazonian basin to determine the relationship between mercury exposure and neurological outcomes and reported the decrease of visual and motor functions with increasing hair mercury levels. In 1998, Lebel and colleagues published another study to determine the neurofunctional and clinical manifestations of nervous system dysfunction in relation to hair mercury levels below 50 ppm. In 1999, Grandjean et al. published results from a study of populations living in four comparable Amazonian riverine communities located upstream of gold-mining fields, while in 2000, Dolbec et al. published results from a cross-sectional study in a village on the Tapajos River.

Lebel et al. (1996)

Lebel et al. (1996) published a study of 29 adult residents living in two villages located on the Tapajos River, a tributary of the Amazon, located approximately 200 kilometers from several gold-mining sites. Total hair mercury concentration ranged from 5.6 to 38.4 ppm; methylmercury constituted between 72.2% and 93.3% of the total mercury measured in hair samples. A quantitative behavioral neurophysiological battery was modified for administration to persons with minimal formal education living in an area without electricity. Women exhibited a decrease in manual dexterity, as measured in the Santa Ana Test (Helsinki version) that was correlated with increased mercury concentration in hair. For both men and women, there was a statistically significant decrease in color discrimination capacity with

increasing hair mercury concentrations. Near visual contrast sensitivity profiles and peripheral visual field profiles were both reduced in the individuals with the highest hair mercury concentrations. The authors note that constriction of the visual field has been observed in other instances of mercury intoxication and that changes in contrast sensitivity have been noted in nonhuman primates exposed to methylmercury (Rice and Gilbert 1982,1990).

Lebel et al. (1998)

A later study was conducted in a Tapajos River village that depends on fish as its main source of protein. A total of 91 adults (45 men and 46 women between the ages of 15 and 81) of the 98 voluntary participants were examined. Four measures of hair mercury concentrations were used: (1) mean total hair mercury, (2) total hair mercury, (3) total hair mercury in the highest value obtained out of all centimeters analyzed, and (4) total hair mercury in the first centimeter and methylmercury in the first centimeter. Several tests were administered to score for neuropsychological dysfunction. Motor strength was determined with a dynameter for grip test; manual dexterity was measured with the Santa Ana Test (Helsinki version); and visual functions, color vision, and contrast sensitivity were assessed with a battery of sensitive neurofunctional tests. Results were analyzed by multiple regression.

There was no difference between genders for all tests except the grip strength test. Women also exhibited decreased grip strength with increasing peak mercury levels. Intermediate and higher frequencies of near visual contrast sensitivity and manual dexterity (measured with the Santa Ana Test) varied with the level of mercury in hair. Gender-nonspecific muscular fatigue was also noted with increasing mercury levels. The authors suggest that there appears to be a dose-effect relationship for certain motor and visual functions. Manual dexterity, alternating hand coordination, and muscular fatigue were associated with hair mercury levels, while near visual contrast sensitivity and restricted visual fields were dose-dependently altered.

Cross-Sectional Study (Grandjean et al., 1999)

A cross-sectional study was conducted in four comparable Amazonian riverine communities located upstream toward gold-mining fields. Fish is consumed as a large part of the population's staple diet. Of the 420 eligible children between the ages of 7 and 12, 351 were examined for neurobehavioral dysfunction. Mercury exposure was measured through children's hair mercury levels because only 37% of the participants had maternal hair mercury samples. Children's hair mercury concentrations had an

overall geometric mean of 11.0 ppm and a median of 12.8 ppm, while mothers had geometric mean hair mercury levels of 11.6 ppm and a median value of 14.0 ppm. Maternal hair mercury concentrations were highly correlated with those of their children. Several neuropsychological tests of motor function, attention, and visuospatial capability were administered. These included Finger Tapping, Santa Ana form board, WISC-III Digit Spans Test, and two subtests of the Stanford-Binet Intelligence Scale (the copying test and memory condition). The relation between mercury exposure and neurobehavioral function was analyzed by multiple regression analyses with adjustment for covariates including, age, sex, health status, maternal education, and maternal marital status.

The Santa Ana form board and Stanford-Binet copying test showed the clearest associations with the hair mercury concentration. The authors note that the effect of mercury was significantly greater in younger children only for the nonpreferred hand condition of the Santa Ana Test. In interpreting these results, the authors caution that there were no data for the level of prenatal exposure experienced in the test children because of the lack of maternal hair samples. Additional sources of uncertainty in this study include nutritional deficiencies that occurred in the past and possible infection of tropical diseases that may have influenced the capabilities of these children at the time of neurological evaluation.

Cross-Sectional Study (Dolbec et al., 2000)

A cross-sectional study was conducted in May of 1996 in a village on the banks of the Tapajos river in the Amazonian Basin, Brazil (Dolbec et al., 2000). This study was conducted on 84 fish-eating adults between the ages of 15 and 79, to evaluate the effect of mercury exposure on motor performance. The mean hair total mercury level was 9 ppm. Pychomotor performance was evaluated using the Santa Ana Test for manual dexterity, the Grooved Pegboard Fine to test fine motor skills and NES Finger Tapping Test for motor speed. Motor strength was measured by dynamometry for grip and pinch strength.

Multivariate analysis of the variance indicated that the hair mercury levels were inversely associated with overall performance on the psychomotor tests, whereas an association was reported with blood mercury. Semipartial regression analyses reported that hair total mercury accounted for 8%-16% of the variance of psychomotor performance. The authors conclude that the findings of this study demonstrated neurobehavioral manifestations of subtle neurotoxic effects on motor functions associated with low-level methylmercury exposure.

3.2.1.11 Madeira

Cross-Sectional Study (Murata et al., 1999b)

A cross-sectional study (Murata et al., 1999) was conducted in the Madeiran community to determine possible—mercury exposure-related effects on evoked potentials in 149 children between the ages of 6.4 and 7.4 years. Children's hair mercury concentrations were used to reflect current exposure levels, while maternal hair levels from mothers who had followed consistent diets since pregnancy represented prenatal mercury exposures 7 years ago. The use of maternal hair concentration as a substitute for exposure during pregnancy is based on the assumption that mercury exposure has changed very little over time. The authors acknowledge, however, that current maternal hair mercury levels provide an imprecise indication of exposure during pregnancy and any recent dietary change would tend to weaken the association with the outcome variables. The 149 children were administered physical and functional neurological examinations, with an emphasis on motor coordination and perceptual motor performance. Tests included these:

- NES2 FT
- NES2 HEC
- NES2 CPT
- WISC-R subtests: Digit Spans forward condition and Block Designs
- Stanford-Binet Bead Memory Test

Evoked potentials were determined with a four-channel electromyograph, while pattern reversal visual-evoked potentials with binocular full-field stimulation were conducted in a darkened room. Associations between these outcomes and exposure to methylmercury were assessed by multiple regression analysis and were adjusted for possible confounding variables: age, sex, maternal and paternal education and employment, maternal alcohol use and smoking during pregnancy, numbers of older and younger siblings, school, and the level of the child's computer acquaintance.

Increased exposure to methylmercury was associated with delays in evoked potential latencies; peak III on the BAEP at 40 Hz, and N145 on the pattern reversal visual-evoked potentials at the 15-minute condition. When the maternal hair mercury concentration exceeded 10 ppm, the increase of the N145 visual-evoked potential latency at 15 minutes was 3.16 milliseconds (ms). The N75-N145 and P100 and N145 interval latencies showed similar regression coefficients for mercury, although

significance was evident only for the 15-minute condition. The authors suggest that this may indicate that there is a mercury-associated delay occurring between P100 and N145. Weak associations were also evidenced between maternal hair mercury levels and deficits on Digit Spans and Bead Memory tests.

3.2.1.12 French Guiana

Case-Control Study (Cordier and Garel, 1999)

High-exposure areas were selected in the Amerind villages in the Upper Maroni, with two other Amerind villages with less mercury contamination to serve as reference groups (Cordier and Garel, 1999. 261 children participated in the study, 69 from the village of Camopi (control), 82 from Awala (control) a total of and 110 in the Upper Maroni (cases). Hair samples were collected from both children and mothers to represent exposure indices. Maternal hair mercury levels ranged from 2.5 to 6.7 ppm. This was used as a surrogate for prenatal exposure. Children had slightly lower hair mercury levels than adults, but this did not vary with age. Neurological examinations were administered to children from 9 months to 6 years of age with special emphasis on neuromotor examination of the upper and lower limbs, axis of the body, deep reflexes, postural reactions, examination of the effects on neuromotor functions, neurosensory examination, and cranial growth. The battery of tests was selected to measure the child's abilities outside of educational or cultural influences; these include the NES FT Test to measure fine motor function, coordination, and speed of execution; and the Stanford-Binet Intelligence Scale, with subtests of immediate memory (bead memory) and ability to assess visuospatial and visuoconstructional function (block-copying). In addition, the McCarthy memory test for digits (backward and forward) and the McCarthy leg coordination test were utilized. Associations were analyzed by linear regression, adjusting for potential confounding factors (alcohol consumption during pregnancy, parity, place of birth of the child, and illnesses during childhood).

Within the case group, there is a significant decrease in the scores with exposure category for the Leg Coordination test and close to significance for the Copying test. When boys and girls were examined separately for the FT test, boys had higher scores than the girls, while a significant decrease is observed in the score on the Block Design test correlated with exposure in girls. Boys also exhibited greater incidence of increased reflexes correlated with maternal hair mercury concentrations. The authors conclude that results of this study suggest a link between exposure to mercury and perturbations of the child's neurological and intellectual development.

3.2.2 Animal Studies

Substantial information on the neurotoxicity of methylmercury has been generated from animal studies that support neurological effects reported in humans. Relatively brief, high-level exposures in rats have been shown to cause characteristic signs of neurotoxicity (flailing and hindlimb crossing when the animal is lifted by the tail), as well as neuronal degeneration in the cerebellum, cerebral cortex, and dorsal root ganglia (Inouye and Murakami, 1975; Leyshon and Morgan, 1991; Magos et al., 1985; Yip and Chang, 1981). As observed in humans, there is a latency period before onset of neurological symptoms. Toxic effects may not be observed or may not show maximal severity until several days after the initiation of dosing. In short-term studies, toxicity may not become evident until after the cessation of dosing. This section summarizes a few selected animal studies on neurotoxicity. For additional detail, please refer to Volume V of the *MSRC* (U.S. EPA, 1997e) and the *Toxicological Effects of Methylmercury* (NRC, 2000).

3.2.2.1 Acute Toxicity

In an acute study, exposure of rats to a single gavage dose of 19.9 mg mercury/kg as methylmercuric chloride resulted in impaired open-field tests such as decreases in standing upright, area traversed, and activity compared with the control group (Post et al., 1973). Animals were lethargic and ataxic initially, but symptoms disappeared within 3 hours.

3.2.2.2 Chronic Toxicity

Longer term, low-level exposures revealed that evidence of neuronal degeneration may be observed before the onset of overt signs of toxicity. Degeneration in the cerebellum was found in rats given 10 mg mercury/kg as methylmercuric chloride once every 3 days for 15 days (Leyshon and Morgan, 1991). Severe degenerative changes in the dorsal root fibers were observed in rats given 1.6 mg mercury/kg-day as methylmercuric chloride for 8 weeks (Yip and Chang, 1981). Munro et al. (1980) observed demyelination of dorsal nerve roots and damage in sciatic nerves with oral exposure to 0.25 mg mercury/kg-day as methylmercuric chloride for up to 26 months. In mice given 1.9 mg mercury/kg-day as methylmercury, cerebellar lesions were observed as early as 8 days after the start of dosing, but changes in motor activity did not develop until after 24 weeks of exposure (MacDonald and Harbison, 1977). Similarly, cats receiving methylmercury in the diet for 11 months displayed degenerative changes

in the cerebellum and cerebral cortex, but uncoordinated movements or weakness were observed only in a small number of animals with histopathological changes (Chang et al., 1974).

A 2-year feeding study of methylmercuric chloride was conducted in B6C3F1 mice (60 mice/sex/group) at doses of 0, 0.4, 2, and 10 ppm (0, 0.03, 0.15, and 0.73 mg mercury/kg-day in males; 0, 0.02, 0.11, and 0.6 mg mercury/kg-day in females) to evaluate chronic toxicity and carcinogenic effects (Mitsumori et al., 1990). Mice were examined clinically during the study, and neurotoxic signs characterized by posterior paralysis were observed in 33 males after 59 weeks and in 3 females after 80 weeks in the 0.6 mg mercury/kg-day group. A marked increase in mortality and a significant decrease in body weight gain were also observed in the high-dose males, beginning at 60 weeks. Postmortem examination revealed toxic encephalopathy consisting of neuronal necrosis of the brain and toxic peripheral sensory neuropathy in both sexes of the high-dose group. An increased incidence of chronic nephropathy was observed in the 0.11- and 0.6-mg mercury/kg-day males.

Groups of Wistar rats (50/sex/group) were administered daily doses of 0.002, 0.02, 0.05, and 0.25 mg mercury/kg-day as methylmercuric chloride for 26 months (Munro et al., 1980). Female rats that received 0.25 mg/kg-day had reduced body weight gains and showed only minimal clinical signs of neurotoxicity. Male rats that received this dose did show overt clinical signs of neurotoxicity, had decreased hemoglobin and hematocrit values and reduced weight gains, and showed increased mortality. Histopathologic examination of rats of both sexes receiving 0.25 mg/kg-day revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe kidney damage and females had minimal renal damage. This study identified a NOAEL of 0.05 mg/kg-day and a LOAEL of 0.25 mg/kg-day, based on the observed demyelination effect.

Bornhausen et al. (1980) reported a decrease in operant behavior performance in 4-month-old rats whose dams had received methylmercuric chloride on gestation days 6 to 9. A statistically significant effect was seen in offspring whose dams had received 0.01 and 0.05 mg/kg five times during gestation. The authors postulated that more severe effects of *in utero* exposure would be seen in humans because the biological half-life of mercury in the brain of humans is five times longer than in the rat. In addition, much longer *in utero* exposure to mercury would occur in humans because gestation is much longer.

In a study of prenatal coexposure to metallic mercury vapor and methylmercury and their effects on the developing central nervous system, Fredriksson et al. (1996) reported interactive behavioral effects following exposure of pregnant female Sprague-Dawley rats to methylmercury and metallic mercury

vapor. Between 4 and 5 months, testing of behavioral function, spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food were performed. Exposure to mercury vapor at 1.8 mg/m^3 for 1.5 hours per day on gestation days 14 to 19 was related to hyperactivity and decreased spatial learning. Although exposure to methylmercury at 2 mg/kg per day on gestation days 6 to 9 was not related to adverse behavioral effects, coexposure to methylmercury and mercury vapor potentiated the activity and spatial learning effects observed with mercury vapor alone. The results of this study indicate that mercury vapor causes central nervous system functional disturbances in offspring after both prenatal and postnatal exposure. The authors also suggest that coexposure to methylmercury served to significantly aggravate the changes, whereas methylmercury alone did not cause any significant functional alterations in this study.

Ramussen and Newland (1999) studied the acquisition of Multiple Differential Reinforcement of High-Rate Extinction (MULT DRH-N:T EXT) schedules of reinforcement in female rats exposed to methylmercury during development. Female rats were administered methylmercury (0, 0.5, or 6.4 ppm) in drinking water from 4 weeks premating to postnatal day 16. Postnatal methylmercury concentrations in the brain at birth were 0.49 and 9.8 ppm for two exposure groups. In the MULT DRH-N:T EXT, female offspring were trained to press levers under schedules of reinforcement. Whenever a response occurred within a specific time measured in seconds, a food pellet was given. Two acquisition protocols were examined; one imposed three successive sessions in a 3:1, 5:2, and 9:4 ratio. Values were chosen so that the same rate of response was required by the schedules. The second acquisition protocol required lever repressing as reestablished and the three schedules were continued until the behavior became stable, which required more than 10 sessions. This study was not able to replicate the finding of abnormal response patterns using the DRL paradigm used by Bornhausen (1980).

Cholinergic systems also play an important role in learning and memory. Coccini et al. (2000) investigated the effect of low-level methylmercury exposure on muscarinic cholinergic receptor (mAChR) binding characteristics in adult female Sprague-Dawley rats. The rats (4/dose) were administered methylmercury in the drinking water at nominal concentrations of 0, 2.5, and 10 µg/L for 16 days. Mean daily intake in the methylmercury-exposed groups was 0.45 and 1.8 mg/kg-day, respectively. mAChR binding was assessed using the muscarinic antagonist [^{3}H]quinuclidinyl benzilate (QNB) to label receptors in excised brain tissues (cerebral cortex, hippocampus, and cerebellum). Exposure to methylmercury selectively increased mAChR density in the hippocampus and cerebellum by 20% to 44%. This response was characterized by a 2-week latency period before onset. Receptor affinity was

unaffected, as indicated by values for the dissociation constant. No significant effect on mAChR in cerebral cortex was observed.

Nonhuman Primates—Macaca Fascicularis Monkeys

Monkeys appear to be more sensitive to the neurotoxic effects of methylmercury than are rodents. The primate model is particularly useful for studies of developmental exposures because monkeys, like humans, have relatively prolonged periods of gestation, infancy, and adolescence (Burbacher and Grant, 2000). Long-term studies in primates have shown neurological impairment at doses as low as 0.05 mg mercury/kg-day. Exposure of monkeys to 0.03 mg mercury/kg-day as methylmercury for approximately 4 months caused no detectable changes in motor activity or effects on vision or hearing, but degenerative changes were observed in neurons of the calcarine cortex and sural nerve when these were examined by electron microscopy (Sato and Ikuta, 1975). At higher doses (0.08 mg mercury/kg-day), slight tremor, lack of motor coordination, and blindness were observed in monkeys after 4 months of exposure (Burbacher et al., 1988).

Gunderson et al. (1986) administered daily doses of 0.04–0.06 mg mercury/kg as methylmercuric hydroxide to 11 crab-eating macaques *(Macaca fascicularis*) throughout pregnancy. This dosing protocol resulted in maternal blood levels of 1,080–1,330 µg/L in mothers and 1,410–1,840 µg/L in the offspring. Infants of treated mothers exhibited visual recognition deficits when tested 35 days after birth.

Rice (1989b) dosed five cynomolgus monkeys *(Macaca fascicularis*) with 0.05 mg mercury/kg-day as methylmercuric chloride from birth to 7 years of age. Clinical and neurological examinations were performed during the dosing period and for an additional 6 years. Impairment of spatial visual function was observed after 3 years. In the later stages of the observation period, monkeys dosed with methylmercury were clumsier and slower to react when placed in the exercise cage than were unexposed monkeys. Decreased fine motor performance, touch, and pinprick sensitivity, and impaired high-frequency hearing were observed 6-7 years after cessation of dosing (Rice 1989a; Rice and Gilbert, 1982, 1990).

Rice (1998) did auditory testing of *Macaca fascicularis* monkeys exposed to methylmercury chloride at 10, 25, or 50 ug/kg per day *in utero*, throughout gestation, plus 4 years postnatally at 11 and 19 years of age. Results from this study indicated that at 19 months of age, all five *Macaca fascicularis* monkeys experienced deterioration in auditory function and elevated pure-tone thresholds throughout the

full range of frequencies tested (0.125 to 31.5 kHz) when compared with age-matched controls. The elevation of thresholds was in some cases 50 dB or higher. Because the auditory deficits are experienced approximately 7 to 15 years after cessation of methylmercury exposure, they are considered irreversible and permanent. The author concluded from this study that the high-dose monkeys experience an earlier onset of effect on the auditory function than do low-dose monkeys. The group of monkeys that showed delayed neurotoxicity at 15 years also had visual deficits identified at 3 years, as well as auditory and somatosensory impairment. The high-dose monkeys were also impaired at 11 years, and relatively more impaired than controls at 19 years, thus providing evidence for accelerated aging. These results provide evidence for the accelerated impairment of auditory function during aging as a consequence of developmental methylmercury exposure.

In another study by Rice (1998), monkeys with robust methylmercury-induced deficits in visual, auditory, and somatosensory function were tested on a series of tasks assessing central processing speed. This task is thought to be similar to tests measuring human intelligence. Five *Macaca fascicularis* monkeys were dosed with 50 μg/kg per day methylmercuric chloride from birth until 7 years of age. Blood mercury levels ranged from 0.8 to 1.1 μg/g until cessation of dosing. At 20 years of age, the monkeys and four age-matched and rearing-matched controls were tested on a series of simple and complex reaction-time tasks. In the simple reaction-time test, the monkeys were required to press a button when it changed from off to on (bright red light). The monkeys then performed a sequence of complex reaction-time tasks: two-button pressing, four-button pressing, and several tasks of increasing complexity using four buttons and multiple colors. The results indicated no differences between groups on any aspect of the experiment. The author concluded that the data provide further evidence for the absence of cognitive impairment in monkeys exposed developmentally to methylmercury.

In 1999, Burbacher et al. published a study that assessed visual and auditory functions in adult *Macaca fascicularis* monkeys exposed to methylmercury *in utero*. Maternal doses were 0, 50, 70, or 90 μg/kg per day; this resulted in infant blood mercury levels that ranged from 1.04 to 2.45 ppm. When the monkeys reached 15 years of age, they were tested on spatial visual contrast sensitivity tasks at spatial frequencies of 1, 4, 10, and 20 cycles per degree of visual angle and auditory pure tone detection tasks at frequencies of 125, 500, 1,000, 4,000, 10,000, 25,000, and 31,500 Hz. The results of these tests indicated that *in utero* exposure to methylmercury has long-term effects on visual contrast sensitivity thresholds. Preliminary results from the auditory task suggest that auditory thresholds are not affected by methylmercury exposure. The authors suggest that results from this study point to the postnatal period as a possible critical window for methylmercury induced auditory neurotoxicity.

3.3 CARDIOVASCULAR TOXICITY

3.3.1 Human Studies

3.3.1.1 Cardiovascular Effects From the Faroe Islands (Sorensen et al., 1999)

Sørensen et al. (1999) evaluated the relationship between prenatal exposure to methylmercury and occurrence of cardiovascular effects at 7 years of age in a birth cohort (n = 1,000) of children from the Faroe Islands. Prenatal exposure was assessed by analysis of cord blood and maternal hair collected at parturition. More than 80% of the hair samples exceeded a methylmercury concentration of 2 ppm, which corresponded to a cord blood concentration of approximately 10 µg/L. The cardiovascular endpoints evaluated at 7 years included systolic and diastolic blood pressure, heart rate, and heart rate variability. Weight, height, body mass index, sex, and maternal hypertension were examined as predictors of blood pressure and heart rate in approximately 900 children. Birth weight and placental weight were also examined as predictors of blood pressure. Following adjustment for body weight, diastolic and systolic blood pressure increased by 13.9 mm mercury (95% confidence limits [CL] = 7.4, 20.4) and 14.6 mm mercury (95% CL = 8.3, 20.8), respectively, as cord blood mercury concentrations increased from 1 to 10 µg/L. No further increase was noted at higher concentrations of mercury. Low-birth-weight children were more likely to experience methylmercury-related increase in blood pressure. A gender-specific decrease in heart rate variability was also noted with increasing mercury exposure. This effect was most pronounced in boys, where a 47% reduction in heart rate variability was observed when cord blood mercury concentrations increased from 1 to 10 µg. The authors concluded that the findings suggest that prenatal exposure to methylmercury may influence the development of cardiovascular regulatory mechanisms.

3.3.1.2 Cross-Sectional Study (Salonen et al., 1995)

Salonen et al. (1995) examined the relationship between dietary intake of fish and mercury and risk of acute myocardial infarction (AMI), death from coronary heart disease (CHD), and other cardiovascular diseases (CVD). Participants of this study included 1,833 men in eastern Finland between the ages of 42 and 60 with no clinically diagnosed CHD, claudication, stroke, or cancer. Baseline examinations were administered between March 1984 and December 1989. Fish consumption was assessed at time of blood sampling with an interview-verified 4-day food record. The food recording was repeated approximately 12 months after the baseline examination in a random sample of 50 men in the

cohort. Daily fish intake ranged from 0 to 619.2 g (mean of 46.5 g/day). Mercury in hair and urine was determined by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation. Hair mercury concentrations ranged from 0 to 15.67 ppm (mean of 1.92 ppm) while dietary mercury intake ranged from 1.1 to 95.3 µg /day (mean of 7.6 µg per day). In 2 to 7 years, 73 of the 1,833 men experienced an AMI; 18 of the 73 patients with AMI died of CHD, while 24 of the 73 died of CVD. Covariates included these: age; examination year; family history of CHD; place of residence (rural vs. urban); diabetes; socioeconomic status; iron intake; number of cigarettes, cigars, and pipefuls of tobacco currently smoked daily; duration of regular smoking in years; alcohol consumption; history of myocardial infarction; angina pectoris and other ischemic heart disease; presence of hypertension; and current antihypertensive medication. The Cox models reported dietary intakes of fish and mercury associated with increased risk of AMI and death from CHD, CVD, and any death. Results from this study indicated that eastern Finnish men with hair mercury levels exceeding 2 ppm had a twofold age- and CHD-adjusted risk of AMI and a 2.9-fold adjusted risk of cardiovascular death compared with those having lower hair mercury content.

3.3.1.3 Nested Case-Control Study (Salonen et al., 1995)

A nested case-control study was also conducted using a subsample of the original study participants. Serum immune complexes containing oxidized LDL were measured in a subsample of 187 control subjects using an ELISA assay with copper-oxidized LDL as the antigen. Pearson correlation coefficients adjusted for age and year of baseline examination were used to determine the association between hair mercury content and dietary intakes of fish and mercury. Partial associations of hair and urinary mercury with titers of immune complexes against oxidized LDL were estimated by SPSS step-up least-squares regression analysis. A multivariate logistic model included the following covariates: cigarette-years, serum ferritin concentration, ischemic exercise ECG, serum apolipoprotein, family history of CHD, maximal oxygen uptake, and serum HDL2 cholesterol. There was a statistically significant association between urinary mercury excretion and the risk of AMI was reported. For each microgram of mercury excreted daily, the risk of AMI increased by 36%. From the immunotoxicity test, both the hair and urinary excretion mercury levels were associated with immune complex titers measured with a rabbit antiserum against oxidized LDL and the γ-globulin fraction of a rabbit antiserum against oxidized LDL. Overall, hair mercury was the strongest predictor of both immune complex titers.

On the basis of these data, the authors concluded that a high intake of mercury from nonfatty freshwater fish, and the consequent excess risk of AMI as well as death from CHD and CVD in eastern Finnish men, may be due to the promotion of lipid peroxidation by mercury.

3.3.2 Animal Studies

Data on cardiovascular effects following oral methylmercury exposure were obtained from two studies in rats. Rats given two daily doses of methylmercuric chloride exhibited decreases in heart rates following two daily doses of methylmercury at 12 mg/kg per day (Arito and Takahashi, 1991). Wistar rats (n = 80) treated by subcutaneous injection with 0.5 mg/kg-day methylmercuric chloride for 1 month had increased systolic blood pressures beginning 42 days after cessation of dosing (Wakita, 1987). This effect persisted for more than a year.

Mitsumori et al. (1983, 1984) fed Sprague-Dawley rats diets containing methylmercuric chloride (males 0, 0.011, 0.05, or 0.28 mg/kg/day; females 0.014, 0.064, or 0.34 mg/kg/day) for up to 130 weeks. Polyarteritis nodosa and calcification of the arterial wall were seen at the highest dose. Histological examination revealed evidence of hemosiderosis and extramedullary hemotopoiesis of the spleen.

In a study on 7-week-old, hypertensive SHR/NCrj rats, Tamashiro et al. (1986) reported an increase in blood pressure resulting from exposure to methylmercury chloride once a day at 2 mg/kg/day for 26 consecutive days. Body weight loss, an early sign of methylmercury intoxication, was more marked in males than females. All male rats died by the 29th day posttreatment. Neurological signs, hindleg crossing, disturbed righting movement and abnormal gait always preceded death. No mortality was reported for the female rats. However, increase in blood pressure was sex-specific, being observed only in females. The authors noted that considerable variation was observed in blood pressure for both the methylmercury-exposed and the control rats; and that these findings suggest strain differences in male-female toxicity of methylmercury chloride.

3.4 IMMUNOTOXICITY

3.4.1 Human Studies

At this time, there are no studies published on the effect of methylmercury on the human immune system. In occupational exposure studies, elemental mercury has been found to affect particular immune

parameters. A study by Queiroz and Dantas (1997) evaluated B-lymphocyte, T-helper, T-suppressor, and T-cell proliferative response to phytohemagglutinin in 33 male workers in a Brazilian mercury production facility. These workers had a mean age of 29 and a mean mercury exposure period of 19 months. All of the workers had urinary mercury concentrations below 50 µg/g of creatinine. Analysis of the T-cell populations found a reverse CD4+ to CD8+ ratio that was characterized by a reduction in the number of CD4 lymphocytes. B-lymphocytes were also significantly reduced. Analysis of serum antibody levels found increased immunoglobulin E levels but did not detect anti-DNA or anti-nucleolar antibodies. No changes were observed in the proliferative response to phytohemagglutinin of lymphocytes from exposed individuals. The authors reported a negative correlation between the length of exposure to mercury and IgE levels, and no correlations between lymphocyte changes and urinary mercury concentrations, time of exposure, or the age of the workers. (Queriroz and Dantas, 1997)

Another occupational exposure study by Moszczynski et al. (1995) examined the lymphocyte subpopulation of T-cells, T-helper cells, T-suppressor cells, and natural killer cells in the peripheral blood of 81 men exposed to metallic mercury vapors and 36 unexposed men. The average workplace exposure to mercury in air was 0.0028 mg/m^3. Urinary mercury concentrations ranged from 0 to 240 µg/L and concentrations in the blood varied from 0 to 30 µg/L. Stimulation of the T-lymphocytes manifested by an increased number of T-cells, T-helper cells, and T-suppressor cells was observed.

3.4.2 Animal Studies

Data on the potential immunotoxic effects of methylmercury are available from several animal studies. Suppression of humoral and cellular immune responses has been observed in animals after oral exposure to methylmercury or methylmercuric chloride. Decreases in the production of antibody-producing cells and/or decreased antibody titer following inoculation with immune-stimulating agents (such as sheep red blood cells) have been observed in mice and rabbits (Blakley et al., 1980; Koller et al., 1977; Ohi et al., 1976). Decreases in natural killer T-cell activity and reduced thymus weight have been observed in female mice after 14 weeks of exposure to methylmercury (Ilback, 1991). Bernaudin et al. (1981) observed IgG deposits along the glomerular capillary wall of Brown Norway rats treated with methylmercury for 2 months and noted that these deposits were suggestive of autoimmune disease. The following sections include summaries of selected studies.

Wild et al. (1997) evaluated immune function in the offspring of Sprague-Dawley rats exposed to methylmercuric chloride (5 or 500 µg/L) or methylmercury sulfide (5 µg/L) via drinking water. There

were three exposed groups and one control group. The control group was fed plain tap water. Rats of both sexes were treated for 8 weeks prior to mating and treatment of female rats continued throughout pregnancy and nursing. The total duration of indirect exposure of the offspring to methylmercury was 42 days. Immunological function was assessed in six offspring per treatment group at 6 and 12 weeks of age (3 and 9 weeks after termination of methylmercury exposure at weaning, respectively). At 6 weeks, total body weights, splenic weights, and thymic weights were increased in the methylmercury chloride-exposed rats, whereas the rats exposed to methylmercury sulfide experienced only an increase in thymic weight at 6 weeks. At 12 weeks, natural killer cell activity was markedly depressed (56%) for rats exposed to methylmercury chloride in comparison with controls. Methylmercury sulfide appeared to have different effects on the immune system than did methylmercury chloride. For example, the sulfide form affected only thymic weight and had no significant effect on NK or splenocyte cell activity or splenocyte LPR. Whether this result reflects differential distribution of the sulfide form or affinity for different targets in the immune system is unknown. The authors concluded that methylmercury chloride seems to have an effect on splenocytes and natural killer cell activity.

Inorganic mercury has been observed to induce a variety of immune effects in mice. However, until recently there has been limited investigation of the ability of methylmercury to induce similar immune responses. Hultman and Hansson-Georgiadis (1999) investigated the ability of subcutaneously injected methylmercury to induce systemic autoimmunity in five genetically susceptible and resistant strains of mice. Female SJN/L, A.SW, B10.S (H-2^S), BALB/C, DBA/2 (H-2^d), A.TL, and B10.TL (H-2^{tl}) mice were administered subcutaneous injections of 1 mg/kg methylmercury every third day for 4 weeks. This treatment protocol resulted in an average daily dose of approximately 350 µg mercury/kg-day. The immune response to methylmercury differed qualitatively and quantitatively from the response to inorganic mercury. Treatment with methylmercury induced at most a small increase in serum Ig concentrations after 4 weeks of treatment. The observed increases during the treatment period were generally marginal when compared with increases induced by mercuric chloride. Treatment with methylmercury induced development of antinucleolar antibodies (ANoA) targeting the nucleolar protein fibrillarin in the susceptible SJL, A.SW, and B10.S strains. Susceptibility to development of ANoA was linked to the mouse major histocompatibility complex H-2. However, background genes determined the strength of the response in susceptible strains. Serum IgE concentration and ANoA titer increased 2 to 3 weeks after cessation of treatment with methylmercury. In H-2^s mice, methylmercury induced a weaker general (polyclonal) and specific (ANoA) response when compared to mercuric chloride. Unlike mercuric chloride-treated mice, animals administered methylmercury did not develop systemic or renal immune system deposits.

3.5 REPRODUCTIVE TOXICITY

3.5.1 Human Studies

There are no studies of reproductive deficits in humans exposed to low-dose methylmercury.

3.5.2 Animal Studies

There are no two-generation reproductive assays for methylmercury.

3.6 GENOTOXICITY

3.6.1 Human Studies

Data from several studies in humans suggest that ingesting methylmercury may cause chromosomal aberrations and sister chromatid exchanges (SCE) (Skerfving et al., 1970; Wulf et al., 1986; Franchi et al., 1994).

A study of nine Swedish subjects who consumed mercury-contaminated fish and four controls showed a statistically significant rank correlation between blood mercury and percentage of lymphocytes with chromosome breaks (Skerfving et al., 1970). An extension of this study (Skerfving et al. 1974) included 23 exposed (5 females and 18 males) and 16 controls (3 females and 13 males). The authors reported significant correlations between blood mercury level and frequency of chromatid changes and "unstable" chromosome aberrations; there was no correlation with "stable" chromosome aberrations.

The Wulf et al. (1986) study was of 92 Greenlander Eskimos. Subjects were divided into three groups based on intake of seal meat (six times per week; two to five times per week, once a week, or no consumption of seal meat). Higher frequency of SCE in lymphocytes was correlated with blood mercury concentration; an increase of 10 µg mercury per liter of blood was associated with an increase of 0.3 SCE/cell. Positive correlations were also found for smoking, diet, living district, and cadmium exposure.

Franchi et al. (1994) evaluated formation of micronuclei in peripheral blood lymphocytes of Mediterranean fishers, a group with presumed high exposure to methylmercury. Fifty-one subjects were

interviewed on age, number of seafood-based meals/week, and habits such as smoking and alcohol consumption. Total blood mercury was measured; the range was 10.08-304.11 ng/g with a mean of 88.97 ± 54.09 ng/g. There was a statistically significant correlation between blood mercury concentration and micronucleus frequency and between age and micronucleus frequency (U.S. EPA, 1997e)

3.6.2 Animal Studies

In a study with cats (Charbonneau et al. 1976), methylmercury did not induce dose-related unscheduled DNA synthesis in lymphocytes or chromosomal aberrations in bone marrow cells after oral exposure for up to 39 months (Miller et al., 1979). Statistically significant decreases in unscheduled DNA synthesis and increases in chromosomal aberrations were observed, but there was no dose-response.

Strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice (Suter, 1975). When (SEC × $C57B_1$)F_1 males were injected with 10 mg/kg methylmercury hydroxide, there was a slight reduction in the total number of implantations and a decrease in the number of viable embryos. This was not observed when (101 × C3H)F_1 males were exposed in a similar fashion. When female (10 × C3H)F_1 mice were treated with methylmercuric hydroxide, no increase in the incidence of dead implants was observed (unlike the case for mercuric chloride). Changes in chromosome number, but no increase in chromosome aberrations, were observed in oocytes of Syrian hamsters treated with one interperitoneal injection of 10 mg/kg methylmercuric chloride (Mailhes, 1983). Methylmercury was administered subcutaneously to golden hamsters at doses of 6.4 mg or 12.8 mg mercury/kg/body weight. Polyploidy and chromosomal aberrations were increased in bone marrow cells, but there was no effect on metaphase II oocytes. There was an inhibitory effect on ovulation, which the authors noted was not as severe as that induced by mercuric chloride in the same study (Watanabe et al., 1982). Nondysjunction and sex-linked recessive lethal mutations were seen in *Drosophila melanogaster* treated with methylmercury in the diet (Ramel, 1972).

As reviewed in WHO (1990), methylmercury is not a point mutagen but is capable of causing chromosome damage in a variety of systems. In vitro studies have generally shown clastogenic activity but only weak mutagenic activity. Methylmercuric chloride and dimethylmercury were both shown to induce chromosome aberrations and aneuploidy in primary cultures in human lymphocytes; methylmercuric chloride was the more potent clastogen at equally toxic doses (Betti et al., 1992). Both methylmercury and mercuric chloride induce a dose-dependent increase in SCE in primary human

lymphocytes and muntjac fibroblasts; methylmercury was about five times more effective in this regard (Verschaeve et al., 1984; Morimoto et al., 1982).

Methylmercury has been shown to inhibit nucleolus organizing activity in human lymphocytes (Verschaeve et al., 1983). Methylmercury can induce histone perturbation and has been reported to interfere with gene expression in cultures of glioma cells (WHO, 1990). Impaired growth and development was noted in cultured mouse embryonic tissue treated in vitro with methylmercuric chloride, but there was no increase in SCE (Matsumoto and Spindle, 1982). Costa et al. (1991) showed that methylmercuric chloride caused DNA strand breaks in both V79 and rat glioblastoma cells treated in vitro. Methylmercuric chloride produced more strand breaks than did mercuric chloride.

Evidence of DNA damage has been observed in the *Bacillus subtilis* rec-assay (Kanematsu et al., 1980). These authors reported negative results for methylmercury in spot tests for mutagenicity in the following bacterial strains: *E. coli* B/r WP2 and WP2; and *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. Jenssen and Ramel (1980) indicated in a review article that methylmercury acetate was negative in both micronucleus assays and mutagenicity tests in Salmonella; the article referred to Heddle and Bruce (1977) and provided no experimental details. Weak mutagenic responses for methylmercuric chloride and methoxyethyl mercury chloride were observed in Chinese hamster V79 cells at doses near the cytotoxic threshold (Fiskesjo, 1979), and methylmercury produced a slight increase in the frequency of chromosomal nondysjunction in *Saccharomyces cerevisiae* (Nakai and Machida, 1973). Methylmercury, however, caused neither gene mutations nor recombination in *S. cerevisiae* (Nakai and Machida, 1973). Methylmercury retarded DNA synthesis and produced single-strand breaks in DNA in L5178Y cells (Nakazawa et al., 1975).

3.7 CARCINOGENICITY

3.7.1 Human Studies

At this time, no human studies have reported an association between methylmercury exposure and overall cancer rates. Three studies were identified that examined the relationship between methylmercury exposure and cancer. No persuasive evidence of increased carcinogenicity attributable to methylmercury exposure was observed in any of the studies. Interpretation of these studies, however, was limited by poor study design and incomplete descriptions of methodology and/or results.

3.7.2 Animal Studies

The results from three dietary studies in two strains of mice indicate that methylmercury is carcinogenic. Interpretation of two of the positive studies was complicated by observation of tumors only at doses that exceeded the Maximum Tolerated Dose (MTD). Therefore, only one positive animal study is appropriate for consideration. A fourth dietary study in mice, three dietary studies in rats, and a dietary study in cats failed to show carcinogenicity of methylmercury. Interpretation of four nonpositive studies was limited because of deficiencies in study design or failure to achieve an MTD.

Methylmercuric chloride was administered in the diet at levels of 0, 0.4, 2, or 10 ppm (0, 0.03, 0.14, and 0.69 mg Hg/kg-day in males and 0, 0.03, 0.13, and 0.60 mg Hg/kg-day in females) to B6C3F1 mice (60/sex/group) for 104 weeks (Mitsumori et al., 1990). In high-dose males, a marked increase in mortality was observed after 60 weeks (data were presented graphically; statistical analyses not performed). Survival at study termination was approximately 50%, 60%, 60%, and 20% in control, low-, mid-, and high-dose males, respectively, and 58%, 68%, 60%, and 60% in control, low-, mid-, and high-dose females, respectively. The cause of the high mortality was not reported. At study termination, the mean body weight in high-dose males was approximately 67% of controls and in high-dose females was approximately 90% of controls (data presented graphically; statistical analyses not performed). Focal hyperplasia of the renal tubules was significantly ($p<0.01$) increased in high-dose males (14/60; the incidence was 0/60 in all other groups). The incidence of renal epithelial carcinomas (classified as solid or cystic papillary type) was significantly ($p<0.01$) increased in high-dose males (13/60; the incidence was 0/60 in all other groups). The incidence of renal adenomas (classified as solid or tubular type) was also significantly ($p<0.05$) increased in high-dose males; the incidence was 0/60, 0/60, 1/60, and 5/60 in control, low-, mid-, and high-dose males, respectively, and 0/60, 0/60, 0/60, and 1/60 in control, low-, mid-, and high-dose females, respectively. No metastases were seen in the animals. The incidences of a variety of nonneoplastic lesions were increased in the high-dose rats including these: sensory neuropathy, neuronal necrosis in the cerebrum, neuronal degeneration in the cerebellum, and chronic nephropathy of the kidney. Males exhibited tubular atrophy of the testis (1/60, 5/60, 2/60, and 54/60 in control, low-, mid-, and high-dose, respectively) and ulceration of the glandular stomach (1/60, 1/60, 0/60, and 7/60 in control, low-, mid-, and high-dose males, respectively). An MTD was achieved in middose males and high-dose females. High mortality in high-dose males indicated that the MTD was exceeded in this group.

Mitsumori et al. (1981) administered 0, 15, or 30 ppm of methylmercuric chloride (99.3% pure) in the diet (0, 1.6 and 3.1 mg Hg/kg-day) to ICR mice (60/sex/group) for 78 weeks. Interim sacrifices of up to 6/sex/group were conducted at weeks 26 and 52. Kidneys were microscopically examined from all animals that died or became moribund after week 53 or were killed at study termination. Lungs from mice with renal masses and renal lymph nodes showing gross abnormalities were also examined. Survival was decreased in a dose-related manner; at week 78 survival was 24/60, 6/60, and 0/60 in control, low-, and high-dose males, respectively, and 33/60, 18/60, and 0/60, in control, low-, and high-dose females, respectively (statistical analyses not performed). The majority of high-dose mice (51/60 males and 59/60 females) died by week 26 of the study. Examination of the kidneys of mice that died or were sacrificed after 53 weeks showed a significant ($p<0.001$) increase in renal tumors in low-dose males (13/16 versus 1/37 in controls). The incidence of renal epithelial adenocarcinomas in control and low-dose males was 0/37 and 11/16, respectively ($p<0.001$). The incidence of renal epithelial adenomas in control and low-dose males was 1/37 and 5/16, respectively ($p<0.01$). No renal tumors were observed in females in any group. No metastases to the lung or renal lymph nodes were observed. Evidence of neurotoxicity and renal pathology was observed in the treated mice at both dose levels. The high mortality in both groups of treated males and in high-dose females indicated that the MTD was exceeded in these groups.

A followup study to the Mitsumori et al. (1981) study was reported by Hirano et al. (1986). Methylmercuric chloride was administered in the diet to ICR mice (60/sex/group) at levels of 0, 0.4, 2, or 10 ppm (0, 0.03, 0.15, and 0.73 mg Hg/kg-day in males and 0, 0.02, 0.11, and 0.6 mg Hg/kg-day in females) for 104 weeks. Interim sacrifices (6/sex/group) were conducted at 26, 52, and 78 weeks. Complete histopathological examinations were performed on all animals found dead, killed *in extremis*, or killed by design. Mortality, group mean body weights and food consumption were comparable to controls. The first renal tumor was observed at 58 weeks in a high-dose male, and the incidence of renal epithelial tumors (adenomas or adenocarcinomas) was significantly increased in high-dose males (1/32, 0/25, 0/29, and 13/26 in the control, low-, mid-, and high-dose groups, respectively). Ten of the 13 tumors in high-dose males were adenocarcinomas. These tumors were described as solid type or cystic papillary types of adenocarcinomas. No invading proliferation into the surrounding tissues was seen. The incidence of renal epithelial adenomas was not significantly increased in males, and no renal adenomas or adenocarcinomas were observed in any females. Focal hyperplasia of the tubular epithelium was reported to be increased in high-dose males (13/59; other incidences not reported). Increases in nonneoplastic lesions in high-dose animals provided evidence that an MTD was exceeded. Nonneoplastic lesions reported as increased in treated males included the following: epithelial

degeneration of the renal proximal tubules; cystic kidney; urinary cast and pelvic dilatation; and decreased spermatogenesis. Epithelial degeneration of the renal proximal tubules and degeneration or fibrosis of the sciatic nerve were reported in high-dose females.

No increase in tumor incidence was observed in a study using white Swiss mice (Schroeder and Mitchener 1975). Groups of mice (54/sex/group) were exposed from weaning until death to methylmercuric acetate in the drinking water at two doses. The low-dose group received 1 ppm methylmercuric acetate (0.19 mg Hg/kg-day). The high-dose group received 5 ppm methylmercuric acetate (0.95 mg Hg/kg-day) for the first 70 days and then 1 ppm, thereafter, due to high mortality (21/54 males and 23/54 females died prior to the dose reduction). Survival among the remaining mice was not significantly different from controls. Significant ($p<0.001$) reductions in body weight were reported in high-dose males (9–15% lower than controls) and high-dose females (15–22% lower than controls) between 2 and 6 months of age. Mice were weighed, dissected, gross tumors were detected, and some sections were made of heart, lung, liver, kidney, and spleen for microscopic examination. No increase in tumor incidence was observed. This study is limited because complete histological examinations were not performed, and pathology data other than tumor incidence were not reported.

Mitsumori et al. (1983, 1984) conducted a study in Sprague-Dawley rats. They administered diets containing 0, 0.4, 2, or 10 ppm of methylmercuric chloride (0, 0.011, 0.05, and 0.28 mg Hg/kg-day in males; 0, 0.014, 0.064, and 0.34 mg Hg/kg-day in females) to Sprague-Dawley rats (56 animals/sex/group) for up to 130 weeks. Interim sacrifices of 10/group (either sex) were conducted at weeks 13 and 26 and of 6/group (either sex) at weeks 52 and 78. Mortality was increased in high-dose males and females. At week 104, survival was approximately 55%, 45%, 75%, and 10% in control, low-, mid-, and high-dose males, respectively, and 70%, 75%, 75%, and 30% in control, low-, mid-, and high-dose females, respectively (data presented graphically). Body weight gain was decreased in high-dose animals (approximately 20–30%; data presented graphically). No increase in tumor incidence was observed in either males or females. Noncarcinogenic lesions that were significantly increased ($p< 0.05$) in high-dose rats included the following: degeneration in peripheral nerves and the spinal cord (both sexes); degeneration of the proximal tubular epithelium of the kidney (both sexes); severe chronic nephropathy (females); parathyroid hyperplasia (both sexes); polyarteritis nodosa and calcification of the abdominal arterial wall (females); bone fibrosis (females); bile duct hyperplasia (males); and hemosiderosis and extramedullary hematopoiesis in the spleen (males). In addition, mid-dose males exhibited significantly increased degeneration of the kidney proximal tubular epithelium and hyperplasia

of the parathyroid. An MTD was achieved in mid-dose males and in high-dose females; the MTD was exceeded in high-dose males.

No increase in tumor incidence or decrease in tumor latency was observed in another study using rats (strain not specified) (Verschuuren et al., 1976). Groups of 25 female and 25 male rats were administered methylmercuric chloride at dietary levels of 0, 0.1, 0.5, and 2.5 ppm (0, 0.004, 0.02, and 0.1 mg Hg/kg-day) for 2 years. No significant effects were observed on growth or food intake except for a 6% decrease (statistically significant) in body weight gain at 60 weeks in high-dose females. Survival was 72%, 68%, 48%, and 48% in control, low-, mid- and high-dose males, respectively; and 76%, 60%, 64%, and 56% in control, low-, mid- and high-dose females, respectively (statistical significance not reported). Increases in relative kidney weights were observed in both males and females at the highest dose. No effects on the nature or incidence of pathological lesions were observed, and tumors were reported to have been observed with comparable incidence and latency among all of the groups. This study was limited by the small sample size and failure to achieve an MTD.

No tumor data were reported in a study using Wistar rats (Munro, 1980). Groups of 50 Wistar rats/sex/dose were fed diets containing methylmercury; doses of 2, 10, 50, and 250 micrograms Hg/kg-day were fed for 26 months. High-dose female rats exhibited reduced body weight gains and showed minimal clinical signs of neurotoxicity; however, high-dose male rats showed overt clinical signs of neurotoxicity, decreased hemoglobin and hematocrit values, reduced weight gains and significantly increased mortality. Histopathologic examination of the high-dose rats of both sexes revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe dose-related kidney damage, and females had minimal renal damage.

No increase in tumor incidence was observed in a multiple generation reproduction study using Sprague-Dawley rats (Newberne et al., 1972). Groups of rats (30/sex) were given semisynthetic diets supplemented with either casein or a fish protein concentrate to yield dietary levels of 0.2 ppm methylmercury (0.008 mg Hg/kg-day). Another group of controls received untreated rat chow. Rats that received diets containing methylmercury during the 2-year study had body weights and hematology comparable to controls. Detailed histopathologic analyses revealed no lesions of the brain, liver, or kidney that were attributable to the methylmercury exposure. Mortality data were not presented. Interpretation of these data is limited by the somewhat small group sizes and failure to achieve an MTD.

No increase in tumor incidence was observed in a study using random-bred domestic cats (Charbonneau et al., 1976). Groups of cats (4–5/sex/group) were given doses of 0.0084, 0.020, 0.046, 0.074 or 0.176 mg Hg/kg-day either as methylmercury-contaminated seafood or as methylmercuric chloride in the diet for up to 2 years. Controls were estimated to have received 0.003 mg Hg/kg-day. Food consumption and body weight were not affected by treatment with methylmercury. Due to advanced signs of neurotoxicity (loss of balance, ataxia, impaired gait, impaired reflexes, weakness, impaired sensory function, mood change and tremor), cats at the highest dose tested were sacrificed after approximately 16 weeks, and cats at the next highest dose were sacrificed after approximately 54–57 weeks. Cats at the next highest dose generally exhibited mild neurological impairment (altered hopping reaction and hypalgesia). One cat at this dose was sacrificed after 38 weeks because of neurotoxicity, and one cat died of acute renal failure after 68 weeks. Cats at the two highest doses had pathological changes in the brain and spinal cord, but no histopathological changes were noted in other tissues examined. Interpretation of the results of this study is limited because of the small group sizes, early sacrifice of cats at the two highest dose levels and no available data regarding pathological changes in cats at the three lowest dose levels. This study was also limited by its short duration when compared to the lifespan of a cat.

Blakley (1984) administered methylmercuric chloride to female Swiss mice (number/group not specified) in drinking water at concentrations of 0, 0.2, 0.5 or 2.0 mg/L for 15 weeks. This corresponded to approximately 0, 0.03, 0.07 and 0.27 mg Hg/kg-day. At the end of week 3, a single dose of 1.5 mg/kg of urethane was administered intraperitoneally to 16–20 mice/group. No effects on weight gain or food consumption were observed. Lung tumor incidence in mice not administered urethane (number/group not specified) was less than 1 tumor/mouse in all groups. Statistically significant trends for increases in the number and size of lung adenomas/mouse with increasing methylmercury dose were observed; the tumor number/mouse was 21.5, 19.4, 19.4, and 33.1 in control, low-, mid- and high-dose mice, respectively, and the tumor size/mouse was 0.70, 0.73, 0.76 and 0.76 mm in control, low-, mid- and high-dose mice, respectively. The study authors suggest that the increase in tumor number and size may have been related to immunosuppressive activity of methylmercury. It should be noted that this is considered a short-term assay and that only pulmonary adenomas were evaluated.